ENERGY AND GASEOUS METABOLISM OF NORMAL AND DEUTECTOMIZED CHICKS BETWEEN 10 HOURS AND 100 HOURS OF AGE [1]

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SEVEN FIGURES

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OBJECT OF INVESTIGATION

The object of this research was to determine 1) the energy metabolism of chicks of both sexes, both normal and deutectomized,[2] and 2) the effect of temperature of environment on the energy metabolism. Data showing the energy metabolism and the effects of environmental temperature on metabolism are necessary for the elucidation of numerous problems in the field of physiology and nutrition. "At present no question is more important in the physiology and genetics of sex than that of the relation which metabolism bears to sex" (Benedict and Riddle, '29). The effect of the temperature of environment on energy metabolism is of equal or greater importance. The recent summary and analysis of the data for heat production of chickens of both sexes and various ages by Mitchell and Kelley ('33) indicate a notable lack of data for newly hatched chicks.

Measurements of normal chicks under 5 days of age cannot be accepted as measurements of basal heat production since such chicks contain varying quantities of unabsorbed yolk.

[1] Acknowledgment is made to W. H. Burrows of the Animal Husbandry Division, who performed most of the deutectomies, and to Robert Rector, and J. L. Gardiner, also of the Animal Husbandry Division, for their assistance in carrying on the experiments and making the observations.

[2] Chicks from which the unabsorbed yolk has been surgically removed.

Consequently observations on chicks from which the unabsorbed yolk had been removed are necessary.

APPARATUS AND PROCEDURE USED

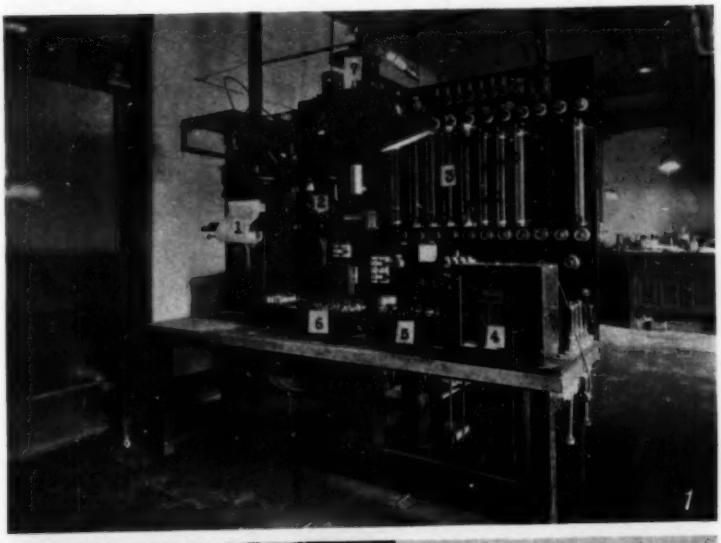
This investigation was conducted by the use of the respiration calorimeter, an instrument for the determination of heat elimination and gaseous exchange, with accurate control of all the physical factors involved. This instrument is described in detail by Barott[3] and by Langworthy and Milner ('15, '16).

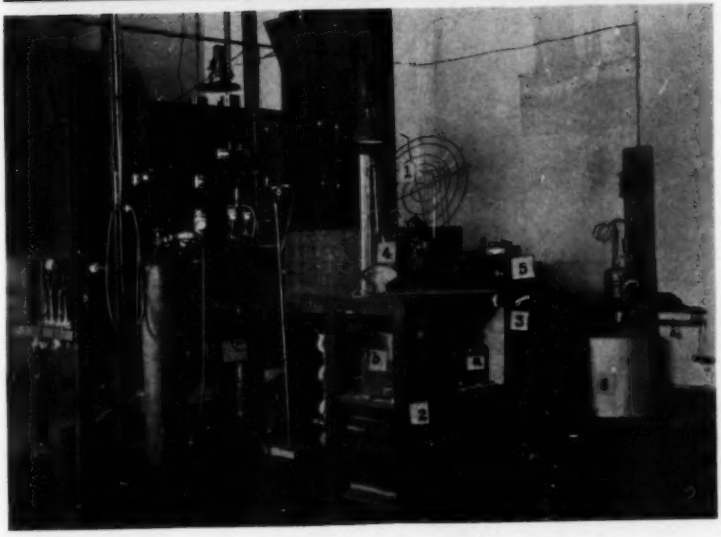
The respiration calorimeter in the form used in this research is a precision instrument, the principles of which may be summarized as follows: for the determination of gaseous exchange the device is a gas-tight chamber connected to a system of gas absorbers in a closed circuit. The gas confined in the circuit is kept in circulation, the gaseous products imparted to it by the experimental birds are constantly removed and oxygen constantly supplied to replace that used. For the measurement of heat produced in the chamber the device is a constant-temperature, continuous water-flow calorimeter, in which the calorimetric features provide for preventing the passage of heat through the walls of the chamber or in the circulating air, and for taking up the heat by a current of water as fast as it is generated in the chamber. The determination of gaseous exchange and heat elimination, to be of value demands a high degree of accuracy in the fundamental measurements and it follows that the instruments with which they are made must be precise and finely adjusted. The calorimeter must be large enough for the purposes of the ex-

[3] Barott, H. G. Effect of temperature, humidity and other physical factors on the chick embryo during incubation. (In course of preparation.)

Fig. 1 Observation table and control board. 1, temperature recorder for water flow to absorber coil; 2, temperature controller for water flow to absorber coil; 3, rheostats to control boundary temperature; 4, controller to control boundary temperature; 5, potentiometer; 6, wheatstone bridge; 7, galvanometer system.

Fig. 2 Calorimetric assembly. 1, absorber coil; 2, absorption train for removing CO_2 and H_2O: a, H_2O absorber flasks; b, CO_2 absorber canister; 3, Crowell blower to provide gas flow through system; 4, standard wet gas meter for metering oxygen; 5, conductivity apparatus for gas analysis; 6, scale and can for weighing water from absorber coil.





periment and yet not so large that its volume will prevent the accurate measurement of the different factors involved. Its walls must be absolutely air-tight, because any leakage through them would nullify the determination of the gaseous exchange and there must be no passage of heat through them

Fig. 3 Calorimeter. 1, outer cork cover; 2, heating coils for control of temperature of boundaries; 3, double copper covers; 4, absorber coil; 5, spirometer to regulate oxygen supply to tension equalizer.

because any transference of unmeasured heat into or out of the chamber would introduce an error into the determination of energy produced within it. Figures 1, 2 and 3 show the assembly of the apparatus.

The calorimeter consists essentially of a double-walled, copper chamber (fig. 3) in the form of a cylinder, 65 cm. in

diameter and 15 cm. deep. The walls are separated by an air space 1 cm. wide in which are inserted a system of differential thermopiles for determining the relative temperature of the two walls. The total volume is approximately 50 liters. The entire top is removable and is hermetically sealed with universal wax.

This double-walled chamber is surrounded by a third boundary of thin insulation board, with an air space 1 cm. thickness separating it from the calorimeter. In this air space are inserted three electric heating coils, uniformly distributed over the surface, one over the top, the second under the bottom and the third around the sides of the outer copper chamber. By means of these coils heat can be supplied to the air space to control the temperature of the outer copper wall. Cork board, 5 cm. thick, on all sides gives further protection against fluctuations in temperature.

GASEOUS EXCHANGE

This copper calorimeter comprises the respiratory chamber. This together with the pump and gas line for circulating the air, and the absorption train for removal of products of respiration, makes up the system, which is of the closed circuit type being completely isolated from the surrounding environment.

The atmosphere of the empty chamber contains oxygen, nitrogen, carbon dioxide and water vapor in proportions like that of ordinary air. When the birds are placed therein, this proportion begins to change because of the consumption of oxygen and the elimination of carbon dioxide and water vapor. The removal of this excess carbon dioxide and water vapor and the restoration of the oxygen used, in such a manner that the quantity may be accurately measured forms the basis of the determination of the respiratory exchange in the chamber. The movement of the air is fixed and constant, once the speed of the pump is adjusted, and the air is uniformly distributed, thus keeping the gas of uniform composition throughout the chamber.

The water vapor and carbon dioxide imparted to the air is removed in specially designed absorbers (fig. 2 a, b). The increase in weight of the absorbers in a given period shows the quantities of these products carried out of the chamber during this period. In a system of this type, as fast as any gas is removed from the air, other gas is introduced to maintain atmospheric pressure in the chamber; in this case, oxygen is supplied automatically. The quantity supplied is accurately metered for the period covering the measurements. The amount of water vapor, carbon dioxide and oxygen measured, corrected for the change in composition of the gas within the chamber, determines the quantity of these products. The amount of carbon dioxide and oxygen in the air of the system is determined by volumetric analysis by use of a Shepherd ('31) gas analysis apparatus; the amount of water vapor is computed from the relative humidity which is measured by a special hair hygrometer.

The volume of air in the chamber varies constantly with the admission of oxygen, the removal of carbon dioxide and water vapor, and with changes of temperature of the air in the chamber and barometric pressure outside. Although this variation is small in magnitude it could result in undesirable variations in the pressure of air in the chamber unless provisions were made for corresponding fluctuations in the capacity of the system. This is accomplished by admitting the oxygen to the chamber through a delicately balanced spirometer which also serves as a tension equalizer.

The absorption train is connected as a shunt off the main gas line, and the volume of air passing through the absorbers is so regulated as to keep the humidity and carbon dioxide content constant.

DETERMINATION OF HEAT PRODUCED

Heat is removed from the calorimeter in two ways: as latent heat of water vapor in the circulating air and as sensible heat liberated to the air by conduction and radiation from the birds. Both latent and sensible heat must be determined.

The water vaporized leaves the chamber in the outgoing air. The quantity of heat leaving the chamber as latent heat of water vapor in any given period is determined by multiplying the weight of water absorbed during the period by the latent heat of water.

The energy eliminated as sensible heat is absorbed by a current of water which circulates through the chamber in the heat absorber coil made of several turns of thin-walled copper tubing. The weight of the water in kilograms that flows through the absorber during a given period, multiplied by the difference of temperature of the water as it enters and leaves the chamber measured by platinum resistance thermometers and converted into degrees Centigrade, represents the amount of heat removed during the period expressed in calories at the mean temperature of the water flowing in the absorber coil.

The rate at which heat is removed from the calorimeter is regulated to prevent fluctuations in the temperature of the air in the chamber. By control of the temperature of the water as it enters the chamber and of the quantity of water which passes through the absorber coil, the removal of heat may be made to accord with its production in the chamber within very narrow limits.

The temperature of the water flowing to the absorber coil is automatically controlled by an electric water heater. The temperature of the walls of the copper chamber and the air within the chamber is determined by means of electric resistance thermometers and by various thermo-couples. The thermal relationship of the two copper walls is determined by a system of thermo-piles kept in equilibrium by the heating coils surrounding the outer wall. The relative temperature of the inflow and outflow air is determined by a differential thermo-pile, one junction of which is inserted in each line at the point of junction with the calorimeter. The temperature of the inflow air is maintained at the same temperature as the outflow by means of a water-cooled heat interchanger and an electric heating coil on the inflow air line.

TESTS FOR ACCURACY OF THE CALORIMETER

At frequent intervals, the accuracy with which measurements can be made with the respiration calorimeter is checked. These checks comprise tests of the apparatus as a calorimeter and as a respiration chamber. To check the instrument as a calorimeter, electric energy is converted into heat in a resistance coil within the chamber, and this heat is measured calorimetrically. The amount produced in a given period of time can be accurately determined from measurements of current flowing in the coil and the voltage drop across the coil. The calorimetric measurements check the electrical to an accuracy of 1 per cent, or in the measurements of very small amounts of energy, by one small calorie.

To check the accuracy of the apparatus as a respiratory chamber, ethyl alcohol is burned in the chamber in such a manner as to insure complete combustion, and the products of combustion consisting of water vapor and carbon dioxide are absorbed and measured. The oxygen used is also measured. These measured amounts are compared with the theoretical amounts computed from the chemical equation for the reactions occurring in the combustion of ethyl alcohol. The results checked to an accuracy of better than 0.5 per cent when 4 gm. of alcohol per hour were burned.

CHICKS USED AND EXPERIMETAL PROCEDURE

The chicks used were of mixed breeding and were obtained from breeding pens kept at the United States Department of Agriculture Research Center, Beltsville, Maryland.

The chicks were placed in wire net boxes with covers. Each box was $5 \times 8 \times 3$ inches in size and was divided into three compartments, each of which accommodated two or three chicks comfortably. The amount of space allowed each chick prevented huddling and also much physical movement.

The effect of temperature variation on energy metabolism was determined at temperatures from 64° to 107°F., with normal and from 68° to 107°F. for deutectomized chicks of both sexes.

For the sex studies normal chicks from one hatch were divided into two lots, one male and one female. The sex was determined by visual examination of the copulatory organs according to the method reported by Jull ('34). After the experiment was completed the chicks were killed for the purpose of verifying the sex. The visual method was shown to have an accuracy of more than 90 per cent. The two groups were placed alternately in the calorimeter for 8-hour periods from the time of sexing to the time when they had reached an age of from 60 to 100 hours. The relative humidity was kept at 60 per cent, the carbon dioxide at less than 1 per cent, and the oxygen at 21 per cent during the observational periods. Between observational periods the chicks were kept in commercial chick boxes at room temperature, the usual manner of holding chicks of their age. Complete measurements were made each 4 hours of the 8-hour period, thus giving two determinations. The chicks were weighed immediately before and after each 8-hour period.

For the experiments involving deutectomized chicks, the unabsorbed yolk in the chick's body at hatching time was readily removed by a simple operation, as reported by Sloan, Card and Adamstone ('34). All deutectomized chicks were allowed at least 8 hours for recovery before being placed in the calorimeter. A few chicks died of hemorrhage within the first 2 hours after operation. The fact that the gram hour rate of metabolism of the operated chicks was proportionately lower than that of the unoperated chicks and that it subsequently dropped regularly with time, is the only evidence available that the recovery period was sufficiently long. The appearance and behavior of the operated chicks was quite normal.

The chicks were divided into three lots: males with yolk sac removed, females with yolk sac removed and normal males used as controls. This gave comparative studies of normal males which have the unabsorbed yolk to live on, and deutectomized males which were deprived at once of all food supply, and a comparison of sexes among deutectomized chicks.

These were placed in turn in the calorimeter beginning with the males used as controls, for 12-hour observational periods. Complete measurements of total heat and gaseous exchange were made every 4 hours during the 12-hour period until the chicks reached an age of from 60 to 100 hours. No feed or water was given at any time after hatch. The chicks were weighed immediately before and after each 12-hour period.

RESULTS FOR NORMAL CHICKS

Figure 4 gives the results on heat elimination and gaseous exchange for normal chicks, in the form of graphs. The results on the normal males used as controls for the deutectomized chicks are added to complete the data obtained. Each point represents the mean value of from four to twelve observations using from twenty-two to thirty-two birds. The effect of variation of temperature is very pronounced. The minimum values occur at approximately 96°F. At this temperature, therefore, is the least expenditure of energy and consequently less food required for basal upkeep.

Mitchell and Haines ('27), reported the critical temperature for Rhode Island Red hens to be about 62°F. but an analysis of their data by the authors shows it to be not lower than 70°F. The critical temperature for the chicks used, under the conditions of the present experiments, was 96°F.

As the temperature varies in either direction the energy metabolism becomes greater until at 89° and 101°F. it has increased nearly 15 per cent. Below 89° the increase is proportional to the decrease in temperature until a temperature of 70°F. is reached, when it is nearly twice as great as at 96°F.

The newly hatched chick is unable to compensate for environmental temperature lower than 70°F. by increasing its own heat production. Chicks permitted to huddle could undoubtedly withstand somewhat lower temperatures.

There is also an appreciable increase in the metabolic rate at temperatures higher than 96°F. At 104°, as the body temperature of the chick is approached, the chick is no longer

able to compensate for high temperatures and the energy metabolism approaches a constant value above this temperature, within the range of temperature studied.

The results in figure 4, which are paired (male and female), were obtained on the same day, one test following the other immediately. All conditions for the two tests were as nearly

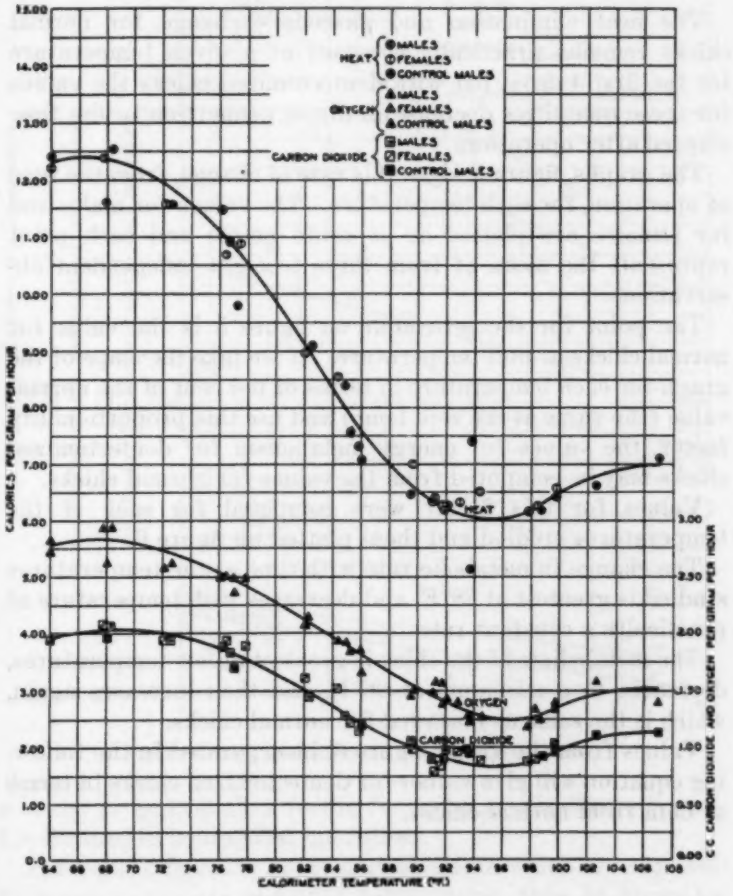


Fig. 4 Heat elimination and gaseous exchange for normal chicks 10 to 100 hours of age.

identical as it was possible to get them. Day and night periods were equally divided among observations on male and female chicks so that the data are not biased by diurnal variations. No consistent difference in energy metabolism between the sexes is shown by these results.

RESULTS FOR DEUTECTOMIZED CHICKS

The heat elimination and gaseous exchange for normal chicks remains practically constant at a given temperature for the first 4 days, but with deutectomized chicks the values for these quantities decrease in direct proportion to the time elapsed after operation.

The graphs, figure 5, show this rate of change, from the time of operation, for each temperature. The values for males and for females are plotted as separate points and each point represents the mean of from three to eight independent observations.

The point for the zero hour on figure 5 is the value for normal chicks at that temperature. If we take the slope of the graph for each temperature in terms of per cent of the normal value (the value at the zero hour) and use this proportionality factor, the values for energy metabolism for deutectomized chicks may be computed from the values for normal chicks.

Values for this factor were computed for each of the temperatures studied and these plotted on figure 6.

The change in metabolic rate with time at the temperatures studied is greatest at 68°F. and decreases with temperature at practically a constant rate.

The metabolism of the chick is greatest at low temperatures, decreases to a minimum at 96°F. and then increases again, which is the same as observed for normal chicks.

Values from the graph, figure 6, incorporated in the following equation will give values for deutectomized chicks in terms of data from normal chicks.

$$Q_d = Q_n at$$

where Q_d is the number of $\left\{\begin{matrix}\text{calories heat}\\ \text{cubic centimeter } CO_2\\ \text{cubic centimeter } O_2\end{matrix}\right\}$ per hour per gram weight for deutectomized chicks. Q_n is the number of $\left\{\begin{matrix}\text{calories heat}\\ \text{cubic centimeter } CO_2\\ \text{cubic centimeter } O_2\end{matrix}\right\}$ per hour per gram weight of normal chicks.

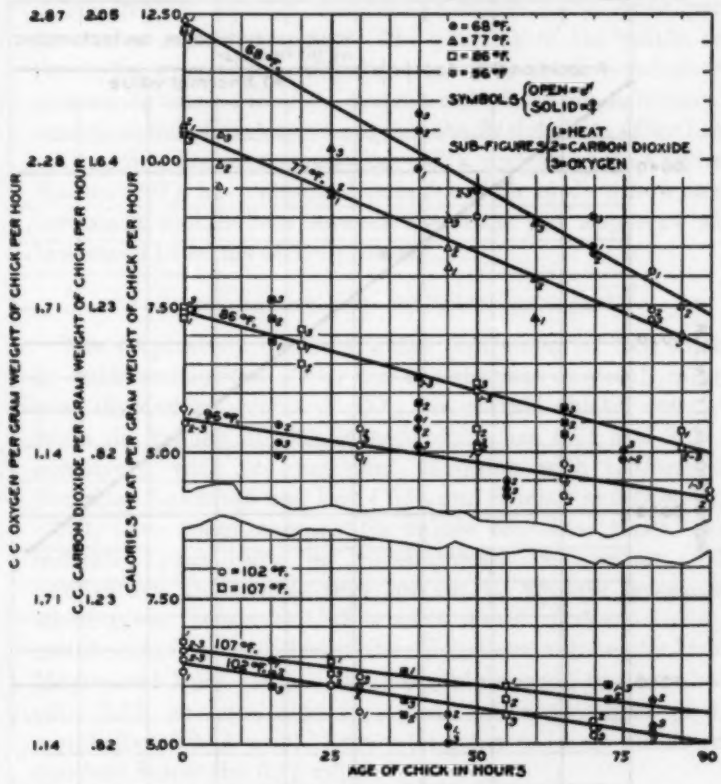


Fig. 5 Energy metabolism of deutectomized chicks.

a is the proportionality factor from the graph, figure 6, and t is the time in hours after operation.

The values, figure 6, apply only to chicks which are exposed 12 hours to the experimental environment, then 24 hours to

brooder environment, followed by another 12 hours to experimental environment, etc.

Values for heat, carbon dioxide, and oxygen for deutectomized chicks may be computed from the above formula. A

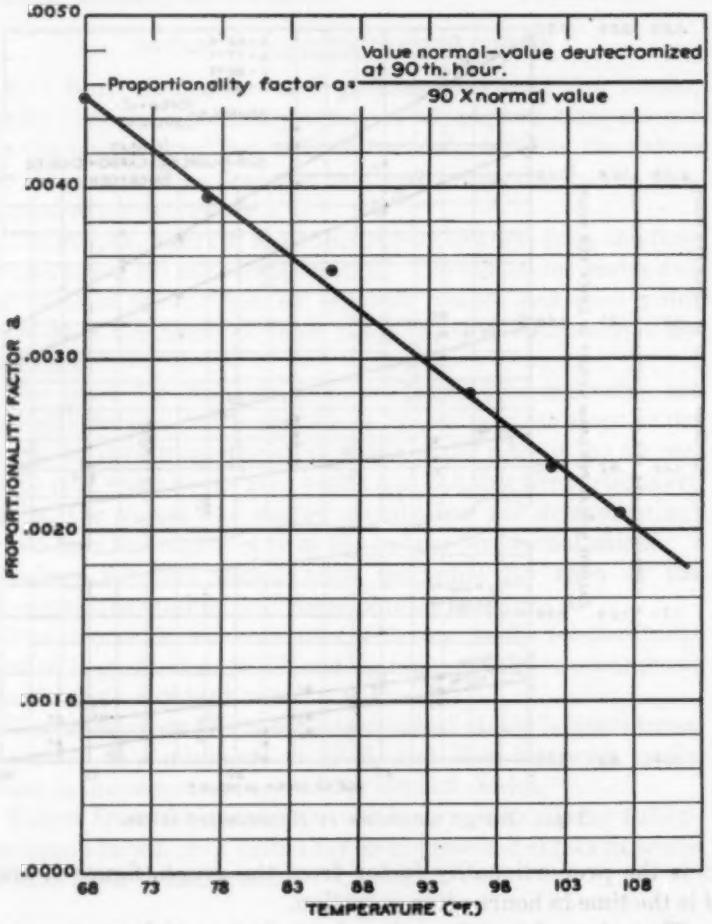


Fig. 6 Graph for computation of values for deutectomized chicks from values for normal.

graph plotted with temperature as abscissa and these values as ordinates will follow that for normal chicks in general characteristics, but each value will of course be less than that for normal chicks by an amount dependent upon the time elapsed after operation.

The graphs, figure 5, for deutectomized chicks show points for males and for females. The position of the points relative to the graph gives an indication of the relative values with respect to sex. All data considered show no difference in energy metabolism due to sex for the first 4 days after hatch.

Benedict, Landauer and Fox ('32), and Mitchell, Card and Haines ('27), have obtained data for older birds where no difference in metabolism between the sexes was apparent when expressed in terms of body weight.

RESPIRATORY AND THERMAL QUOTIENT

The respiratory quotient, cubic centimeter of CO_2 divided by cubic centimeter of O_2, and the thermal quotient, calories heat divided by grams of CO_2, for normal chicks, computed from the results of this investigation, are 0.71 and 3.16 respectively, with no consistent variation with temperature. Benedict, Landauer and Fox ('32), and Kleiber and Dougherty ('34), have found comparable values for older birds. This indicates practically no carbohydrate metabolism. The respiratory quotient is that for fat or protein metabolism which gives the same R. Q. when the end product of protein metabolism is uric acid as in the chick, as was shown by Henry, Magee and Reid ('34). The thermal quotient for fat should give 3.37, so here also is an indication of some protein metabolism which would have a tendency to lower the thermal quotient below the 3.37 value.

The respiratory quotient and the thermal quotient for the deutectomized chicks are the same as for the normal chicks.

LOSS IN WEIGHT OF CHICKS

The rate at which the chick lost weight under the experimental procedure was constant relative to time. It was also

constant relative to temperature below 100°F. except for a slight increase below 80°F., but above 100°F. the rate of loss increased rapidly. This was due, no doubt, to excessive loss of water because of the chick's effort to maintain its body temperature by evaporation when the environmental temperature was approaching its body temperature.

The loss in weight in 3 days in per cent of original weight is as follows:

TEMPERATURE CALORIMETER	NORMAL CHICKS	NORMAL CHICKS LESS CORRECTION FOR YOLK SAC[1]	DEUTECTOMIZED CHICKS
°F.	*per cent*	*per cent*	*per cent*
65– 80	23.5	9.0	26.0
80–100	21.5	9.0	22.0
102	31.0	16.5	28.5
107	42.0	29.5	38.0

[1] The average weight of the normal chicks at the beginning of the experiments was 35.9 gm. and that of the deutectomized chicks 30.5 gm. Thus the average weight of the yolk sac removed was approximately 5 gm. Subtracting this amount from the loss in weight of the normal chick will give the weight of body tissue which was used. This is given as a per cent of the original weight in the above tabulation.

HEAT ELIMINATION AND GASEOUS EXCHANGE PER GRAM LOSS IN WEIGHT OF CHICKS

Figure 7 shows by graphs the heat, carbon dioxide, and oxygen per gram loss in weight of chick for normal chicks for the 3-day period over the range of temperature studied.

The values for gram loss of weight for deutectomized chicks could be computed for any specified time but the values would become lower and lower with time elapsed after operation. They would in all cases be lower than those for normal chicks.

Figure 7 shows the greater efficiency in utilization of body material at the lower temperatures. The heat production per gram loss in weight at 68°F. is more than twice as great as that at 107°F.

The peak in the graph at 98°F. is interesting. The weight loss below 100°F. is very nearly constant, but the energy output at 98° is about 5 per cent greater than at 94°, which causes the graph to rise at this interval. Above 100° the

energy output increases slightly but the rate of loss in weight of the chick increases rapidly thus causing the graph to fall once more.

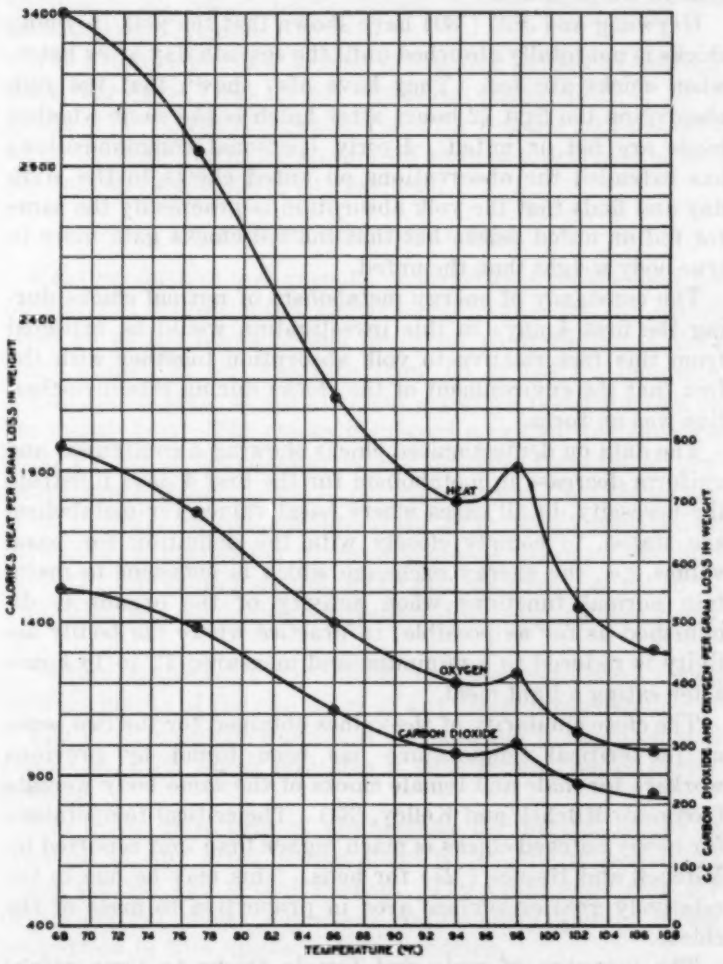


Fig. 7 Heat elimination and gaseous exchange of normal chicks per gram loss in weight.

The data for oxygen consumption of newly hatched chicks presented by Mitchell, Card and Haines ('27), check within 10 per cent those in this paper, at points approximating the critical temperature.

Heywang and Jull ('30) have shown that the yolk in young chicks is not totally absorbed until the seventh day after hatch, when chicks are fed. They have also shown that the yolk absorption the first 72 hours after hatch is the same whether birds are fed or unfed. Byerly (personal communication) has extended the observations on unfed chicks to the sixth day and finds that the yolk absorption is practically the same for fed or unfed chicks but that the fed chicks gain more in true body weight than the unfed.

The constancy of energy metabolism of normal chicks during the first 4 days in this investigation would be expected from this fact relative to yolk absorption together with the fact that the environment of the chicks during this investigation was uniform.

The data on deutectomized chicks showing a continuous and uniform decrease in metabolism for the first 4 days illustrate the necessity, in all cases where basal values for metabolism are stated, to comply closely with the definition for basal values, i.e., the energy exchange which is sufficient to maintain normal functions when activity of the organs is diminished as far as possible; in practice where the bodily activity is reduced to a minimum and measured 12 to 18 hours after eating a light meal.

The close similarity of the values obtained for the two sexes at the critical temperature has been found by previous workers for male and female chicks of the same body weights (compare Mitchell and Kelley, '33). The critical temperature for newly hatched chicks is much higher than that reported by Mitchell and Haines ('27) for hens. This may be due to the relatively greater surface area in proportion to mass of the chicks.

The response of male and female chicks to temperature change is unlike that reported by Riddle, Christman and

Benedict ('30), for ring doves, for their data showed much greater metabolism of the male at lower temperatures than of the female. In the present experiments, values for males and females at all temperatures showed neither appreciable nor consistent differences.

The average decrease in heat production caused by shifting male ring doves from an environmental temperature of 20°C. to one of 30°C. was 28.1 per cent and for females 20.3 per cent, while the male chicks here reported showed a decrease of 39.5 per cent and the females 40.0 per cent.

SUMMARY AND CONCLUSIONS

Heat production and gaseous exchage were determined for normal and deutectomized chicks of both sexes for the period from 10 to 100 hours of age by direct calorimetry. Chicks were studied at temperatures from 68 to 104°F., inclusive.

The temperature of environment showed a decided effect on the metabolism. The chicks displayed a critical temperature at 96°F. Seven degrees increase or decrease from the critical temperature caused about 15 per cent increase in metabolism. With decrease in temperature from 96 to 70°F., metabolism increased in proportion to environmental temperature until at 70°F. the energy output was twice as great as at 96°F. The chick was unable to compensate for temperatures below 70°.

Neither appreciable nor consistent differences were found between chicks of the two sexes at any temperature studied.

The R. Q. observed was about 0.71 and the T. Q., calculated from CO_2, 3.16, which indicates a metabolism free of carbohydrate.

The gram hour rate of metabolism for normal chicks is constant at any given temperature within the physiological range for the age period studied. The gram hour rate for deutectomized chicks decreased continuously and in direct proportion to time elapsed after operation.

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CALCIUM AND PHOSPHORUS RETENTION IN GROWTH, IN RELATION TO THE FORM OF CARBOHYDRATE IN THE FOOD

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Without asking space to review the voluminous literature either of carbohydrate digestion and metabolism or of the economy of calcium and phosphorus in nutrition, we would here record the results of experiments upon calcium and phosphorus retention, in the growing body, when the food, alike in other respects, was systematically varied as to the nature of the carbohydrate which constituted one of the major components of the dietary. The forms of carbohydrate here compared are dextrose (corn sugar, 'cerelose'), spray-dried corn sirup (mixture of dextrose, maltose, and dextrin), dextrin (a commercial edible dextrin made from cornstarch), cornstarch, and sucrose. A series of diets was prepared in each of which one of the dry forms of carbohydrate just named was mixed with twice its weight of the air-dry diet B (diet 13) which has been extensively used in previous investigations in this laboratory (Sherman and Campbell, '24; Sherman and MacLeod, '25; Sherman and Quinn, '26; Sherman and Booher, '31; Fincke and Sherman, '35).

It is known that this diet B, when given alone, supports normal growth and development. Hence its dilution with one-half its weight of carbohydrate was believed to furnish a favorable means of comparing different forms of carbohydrate as to their effect upon calcium and phosphorus retention by the growing and developing organism, especially

if the experimental period were chosen as falling in a segment of the life cycle in which normal development includes a considerable retention of calcium and phosphorus as an essential feature of the normal calcification or ossification of the skeletal system.

EXPERIMENTAL

Young albino rats were transferred at 28 to 29 days of age from the family diet of one-third dried whole milk with two-thirds ground whole wheat (diet B; laboratory no. 13) to experimental diets consisting of two-thirds diet B and one-third of the form of carbohydrate under investigation—dextrose, spray-dried corn sirup, dextrin, cornstarch, or sucrose, respectively—litter-mate controls being continued upon diet B. At 60 days of age, the experimental animals including the controls were killed and analyzed for calcium and phosphorus. The age of 60 days was chosen for this purpose because previous experience of our laboratory with rats of the same colony and nutritional background indicated this to be the age at which the determination would be most conclusive as to whether, on these different experimental diets, calcium (and phosphorus) retention would be alike or different. The data of calcium and phosphorus contents of duplicate rats at the age of 28 to 29 days, as determined both in these experiments and in much related work in this laboratory, make it possible for us to compare the diets characterized by the different forms of carbohydrate here studied, both in terms of the calcium and phosphorus contents of the experimental animals at the age of 60 days and in terms of the amounts of these elements which had been retained during the experimental period.

Calcium was determined by the well-known modified McCrudden method, and phosphorus according to the method of the Association of Official Agricultural Chemists.

In starting the experiments, rats of the same litter were distributed to the different diets in such a way as to afford the fullest practicable measure of litter-mate control in the

comparison of the results obtained upon the different experimental diets with each other, and with those obtained on the control diet (diet B); also to have as even as practicable a distribution of sexes and initial body weights.

The forms of carbohydrate here studied contained from no detectable traces to barely measurable amounts of calcium and phosphorus. Hence all experimental diets contained 0.23 to 0.24 per cent of calcium and 0.32 to 0.33 per cent of phosphorus with a calcium: phosphorus ratio of 0.7. In the light of the previous experience of our laboratory, this appears a sufficiently favorable intake and ratio to constitute a normal nutritional test, yet with the level of calcium intake low enough so as really to put to the test any difference which might exist among the diets containing the different forms of carbohydrate. That such was the case may be seen from the fact that this dilution of the control diet with the carbohydrate studied did result in every case in a slightly less rapid retention of calcium (and phosphorus) than occurred in the control animals which received the undiluted diet B with its 0.35 per cent of calcium and 0.49 per cent of phosphorus and with the same calcium: phosphorus ratio of 0.7. Records of food consumption during the experimental period permit also the approximate estimation of the calcium and phosphorus intakes. The data of these experiments are summarized, for calcium and for phosphorus, respectively in tables 1 and 2.

Rats here analyzed at 28 to 29 days of age showed: for males, 0.68 ± 0.03 per cent calcium and 0.51 ± 0.02 per cent phosphorus; for females, 0.71 ± 0.03 per cent calcium and 0.53 ± 0.02 per cent phosphorus (on the basis of live weight). These percentages are in agreement with the previous experience of this laboratory in like cases, account being taken of the fact that the percentages are expressed sometimes on the basis of live weight and sometimes of net weight as above explained.

TABLE 1

Calcium retentions by rats on diets containing different carbohydrates

ADDED CARBOHYDRATES IN DIET	NUMBER OF RATS	CALCIUM IN FOOD EATEN	NET WEIGHT AT 60 TO 61 DAYS	CALCIUM IN BODY AT 60 DAYS		CALCIUM RETAINED DURING EXPERIMENTAL PERIOD
Males						
		gm.	*gm.*	*gm.*	*per cent of net weight*	*gm.*
Control diet	5	1.077±0.096	148±19	1.169±0.129	0.79±0.02	0.829±0.113
Dextrose	3	0.728±0.066	126±18	0.957±0.153	0.76±0.02	0.613±0.105
Dried corn syrup	4	0.718±0.067	124±10	0.973±0.077	0.79±0.02	0.638±0.061
Dextrin	4	0.661±0.026	114± 8	0.893±0.075	0.79±0.02	0.558±0.046
Cornstarch	3	0.705±0.053	124± 6	0.985±0.051	0.80±0.02	0.625±0.030
Sucrose	4	0.729±0.039	128± 9	0.954±0.054	0.75±0.02	0.629±0.026
Females						
Control diet	4	0.922±0.018	116± 1	1.036±0.014	0.90±0.01	0.708±0.017
Dextrose	3	0.605±0.046	99± 4	0.822±0.027	0.83±0.01	0.509±0.019
Dried corn syrup	5	0.671±0.024	110± 8	0.917±0.051	0.83±0.03	0.574±0.026
Dextrin	3	0.597±0.057	105±10	0.901±0.089	0.86±0.02	0.539±0.065
Cornstarch	3	0.590±0.014	105± 8	0.886±0.072	0.85±0.02	0.530±0.028
Sucrose	4	0.644±0.025	106± 4	0.939±0.053	0.89±0.01	0.581±0.032

TABLE 2

Phosphorus retentions by rats on diets containing different carbohydrates

ADDED CARBOHYDRATES IN DIET	NUMBER OF RATS	PHOSPHORUS IN FOOD EATEN	NET WEIGHT AT 60 TO 61 DAYS	PHOSPHORUS IN BODY AT 60 DAYS		PHOSPHORUS RETAINED DURING EXPERIMENTAL PERIOD
Males						
		gm.	*gm.*	*gm.*	*per cent of net weight*	*gm.*
Control diet	5	1.499±0.125	148±19	0.813±0.116	0.55±0.03	0.558±0.101
Dextrose	3	1.008±0.091	126±18	0.664±0.077	0.53±0.01	0.406±0.042
Dried corn syrup	4	0.970±0.091	124±10	0.691±0.057	0.56±0.01	0.440±0.041
Dextrin	4	0.921±0.041	114± 8	0.633±0.052	0.56±0.01	0.381±0.030
Cornstarch	3	0.976±0.074	124± 6	0.698±0.039	0.57±0.02	0.427±0.022
Sucrose	4	1.010±0.054	128± 9	0.688±0.044	0.54±0.01	0.444±0.023
Females						
Control diet	4	1.290±0.025	116± 1	0.712±0.009	0.62±0.01	0.466±0.011
Dextrose	3	0.843±0.060	99± 4	0.578±0.022	0.58±0.00	0.342±0.012
Dried corn syrup	5	0.906±0.032	110± 8	0.644±0.038	0.58±0.02	0.389±0.020
Dextrin	3	0.827±0.078	105±10	0.633±0.063	0.60±0.01	0.362±0.045
Cornstarch	3	0.816±0.020	105± 8	0.616±0.042	0.59±0.01	0.360±0.017
Sucrose	4	0.913±0.016	106± 4	0.677±0.055	0.63±0.02	0.407±0.034

DISCUSSION OF RESULTS

As contemplated in the plan of investigation above explained, the animals which received the undiluted diet B—the control diet in this case—retained more calcium and phosphorus than those on the experimental diets. Hence it is logical to infer that the experimental diets were such as to constitute a real test of the question whether calcium (and phosphorus) retention procedes with similar or with different efficiency upon diets containing the different carbohydrates here studied.

Also, the animals on the control diet grew somewhat more rapidly than those in the experimental diets, since in the latter the concentration of tissue-building nutrients was diluted by the added carbohydrate. This dilution was, however, quantitatively the same in all cases, so that all these experimental diets contained the same concentrations and ratios; of calcium and phosphorus, and of all other tissue-building nutrients as well, and all were of essentially the same energy or calorie value per gram.

Moreover, the animals receiving the experimental diets grew at rates within the normal range for animals of their age and initial size, and the gains in body weight were essentially alike for all the experimental diets.

The gains in body calcium were also essentially alike on all the experimental rations; as were also the gains in body phosphorus. The small differences in the averages for either calcium or phosphorus retention for animals of either sex on any of the experimental diets appear to lie within the range of probable experimental error. Apparently the retention of calcium (and also of phosphorus) proceded with essentially equal efficiency in the normal growing (mammalian) body whether the dietary (otherwise composed of wheat and milk) contained dextrose (corn sugar, 'cerelose'), spray-dried corn sirup, dextrin, cornstarch, or sucrose as added carbohydrate.

Moreover, the percentages of calcium (and of phosphorus) in the bodies of the animals at the end of the experimental period were essentially alike for those on the control diet and those on the diets diluted with the different carbohydrates.

As would be expected from the greater richness of the control diet in calcium, the animals on this diet used their ingested calcium a little less efficiently than did those on the experimental diets. To compare further the efficiency of utilization on the different experimental diets, the calcium retained divided by the calcium ingested may be shown as a 'calcium utilization factor' as in table 3. Here again the calcium metabolism is seen to be essentially alike under the influence of the different carbohydrates tested, as the small

TABLE 3

'Calcium utilization factors' (ratio of retained to ingested calcium) and ratios of body calcium to body phosphorus as indicated by analyses of experimental animals at 60 to 61 days of age

ANIMALS FROM DIET CONTAINING	MALES		FEMALES	
	Calcium utilization factor	Ca : P ratio	Calcium utilization factor	Ca : P ratio
Dextrose	0.83±0.06[1]	1.4	0.84±0.04	1.4
Dried corn syrup	0.89±0.04	1.4	0.85±0.04	1.4
Dextrin	0.84±0.04	1.4	0.90±0.03	1.4
Cornstarch	0.89±0.03	1.4	0.90±0.02	1.4
Sucrose	0.87±0.05	1.4	0.90±0.02	1.4

[1] Average deviation.

apparent differences in utilization factor are all within the range of experimental error—furthermore the animals on the different diets show the same ratio of body calcium to body phosphorus. Undoubtedly phosphorus retention was here determined by the experimentally restricted intake of calcium which was the limiting factor in the calcification of the growing bodies.

SUMMARY

The experiments here reported were planned to ascertain whether calcium and phosphorus retentions in the normal, growing young mammal are essentially alike or different when different forms of carbohydrate are added to an otherwise identical adequate diet.

In order that the experiments should constitute a sufficiently rigorous test of the question, each form of carbohydrate studied was mixed with twice its weight of the control diet (diet B or diet 13 of this laboratory, a mixture of two-thirds ground whole wheat and one-third dried whole milk; fed with table salt and distilled water). Thus in the experimental diets the tissue-building nutrients were diluted in all cases to the same extent; and to such extent as to make these diets somewhat less than optimal though still adequate to support body growth and skeletal development within the normal range.

Under these conditions it was found that calcium retention (and also that phosphorus retention) was essentially alike whether the carbohydrate was added to the diet in the form of dextrose (corn sugar), spray-dried corn sirup (mixture of dextrose, maltose and dextrin), dextrin, cornstarch or sucrose.

The experimental animals were albino rats bred in the laboratory. Their ages were exactly known, as were the nutritional histories both of the experimental animals and the families from which they came. The periods of experimental feeding began at the age of 4 weeks and were ended by killing and analyzing the animals at the age of 60 days, at which age any difference in calcification, which might have resulted from the difference in form of carbohydrate fed, should have been most readily demonstrable.

Within the limits of experimental error, calcium utilization was alike on the experimental diets containing the different forms of carbohydrate.

The ratio of calcium to phosphorus found in the body was also essentially the same for the animals which had received the different experimental diets.

Moreover, although growth and calcium retention were (designedly, as explained above) somewhat less rapid on the experimental diets than on the control diet, the percentages of calcium and of phosphorus in the bodies at the completion of the test were essentially the same for the animals on the experimental diets as for those of the same age on the control diet.

This latter fact constitutes added evidence that the level of calcium intake provided in the experimental periods was well adapted to the purpose of affording a rigorous test of the efficiency of the normal retention of calcium and phosphorus under the influence of these different diets.

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THE EFFECT OF HEAT AS USED IN THE EXTRACTION OF SOY BEAN OIL UPON THE NUTRITIVE VALUE OF THE PROTEIN OF SOY BEAN OIL MEAL [1]

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Several investigators have reported that the use of heat in the preparation of certain foods definitely alters the nutritive value of the protein. When such materials as casein, meat, liver, kidney, heart muscle, cereals and fish meal were exposed to high temperatures for a considerable time, there resulted a decided decrease in their nutritive value. The reader is referred to a publication by Seegers and Mattill ('35) and by Boas Fixsen ('35) for a discussion of recent studies.

In direct contrast with these results, there is evidence that heating soy beans causes a definite improvement in the growth of animals when they are changed from a raw soy bean diet to a heated soy bean diet. Osborne and Mendel ('17), Vestal and Shrewsbury ('32), and Shrewsbury, Vestal and Hauge ('32) found that ground raw soy beans when fed to rats as the sole or principal source of protein in an otherwise complete ration did not support appreciable growth. However, normal growth resulted when they fed soy beans which had been previously cooked. Vestal and Shrewsbury ('32),

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Shrewsbury, Vestal and Hauge ('32), and Robison ('32) reported similar results with pigs. Mitchell and Villegas ('23), Mitchell and Smuts ('32), McCollum, Simmonds and Parsons ('21), and Shrewsbury and Bratzler ('33) reported experimental evidence in support of the fact that the raw soy bean contains a protein of low nutritive value.

In an attempt to find the reason for the increase in growth brought about by heating the soy bean, Osborne and Mendel ('17) and Vestal and Shrewsbury ('32) conducted digestibility experiments with rats. Both groups of investigators reported that cooking the ground raw soy beans increased the digestibility of the total protein about 4 per cent. They stated that the improved nutritive value of the soy bean caused by heating appeared to be due to an increase in food consumption and nitrogen absorption.

Johns and Finks ('20) working with phaseolin, an isolated protein of the navy bean, found that this protein when cooked, produced markedly better growth, than the raw protein, in the rat. A similar improvement in the growth of the rat resulted when the isolated raw proteins of the velvet bean were fed cooked (Finks and Johns, '21). Cooked phaseolin (Waterman and Johns, '21) and the cooked isolated proteins of the velvet beans (Waterman and Jones, '21) yielded considerable more amino nitrogen when digested in vitro than the raw proteins digested in the same manner.

Although it is common knowledge that heat is used in all the commercial methods of extracting oil from the soy bean, little attention has been given to the effect that the amount of heating used in the process of extraction has upon the nutritive value of the protein in the residue known as soy bean oil meal. With the ever increasing demand for the production of soy bean oil, there arises the problem of using the soy bean oil meal to advantage (Horvath, '33). Apparently, as this meal is destined to be used extensively in rations for livestock and in the dietary of man, information on the effect that the oil extraction temperature has upon the nutritive value of the proteins of soy bean oil meal is important. For

the most part, two methods have been generally used for arriving at protein values; viz., the growth method of Osborne, Mendel and Ferry ('19) and the nitrogen balance method of Mitchell ('24). Since the two values are not comparable (Boas Fixsen, '35), we have employed both methods in the following experiments.

EXPERIMENTAL

Production of experimental material. Commercially, soy bean oil is extracted by the expeller, the hydraulic or the solvent processes. The resulting meals are known according to the method of extraction employed. In addition the hydraulic meal is sometimes spoken of as 'old process meal' and the solvent meal as the 'new process meal.' We included in our experiments these various soy bean oil meals, produced where possible at low, medium, and high temperatures. For the most part they were prepared from beans of the Illini variety. We supervised the production of the samples by the manufacturers and kept a complete history of the various treatments used in their preparation. The data pertaining to the temperatures used are given in table 1. In tables following, the beans used in preparing the expeller meals were designated with the letter A, those used for the hydraulic meals with B, and those used for the solvent meals with C.

Growth experiments. For the growth experiments the ground beans or meal were fed in a basal ration composed of about 50 parts cooked starch, 6 parts yeast, 4 parts of salts no. 40 (Steenbock and Nelson, '23) and 2 parts of cod liver oil. The amount of cooked starch incorporated into each ration was changed with the amount of soy bean material or other supplement required by analysis to supply the desired level of protein. Allowance was also made for any amount of soy bean oil or other ingredients other than the protein supplements specified in the rations.

We employed the ad libitum and equalized types of feeding (Steenbock, Black and Thomas, '30). We used male rats

3 to 4 weeks old at 50 to 60 gm. in weight. They were quartered in cages with raised screen bottoms. In the ad libitum feeding series they were kept in groups of four; in the equalized consumption series, where there were six rats in a group,

TABLE 1

History of preparation of soy bean oil meals[1]

Expeller meals

DEGREE OF HEAT TREATMENT	PRELIMINARY DRYING		CONVEYING		PREWARMING		EXPELLING THE OIL	
	Temperature	Time	Temperature	Time	Temperature	Time	Temperature	Time
	°C.	min.	°C.	min.	°C.	min.	°C.	min.
Low	100–112	8	82–90	7	90	13	Cold shaft 105	2.0
Medium	100–112	8	82–90	7	100–112	13	Hot shaft 112–125	1.5
							Hot shaft 130	1.0
High	100–112	8	82–90	7	100–112	13	Hot shaft 140	1.5
							Hot shaft 150	1.0

Hydraulic meals

DEGREE OF HEAT TREATMENT	PRELIMINARY DRYING		HEATING		COOKING		PRESSING	
	Temperature	Time	Temperature	Time	Temperature	Time	Temperature	Time
	°C.	min.	°C.	min.	°C.	min.	°C.	min.
Low	60–80	60	60	10	82	90	75–65	50–60
Medium	60–80	60	60	10	105	90	75–65	50–60
High	60–80	60	60	10	121	90	80–68	50–60

Solvent meal

PRELIMINARY HEATING		EXTRACTING	DRYING		AERATING AND COOLING	
Temperature	Time	Temperature	Temperature	Time	Temperature	Time
°C.	min.	°C.	°C.	min.	°C.	min.
60	10	45	98	15	78–45	10–20

[1] The expeller meals were prepared in the soy bean plant of Allied Mills, Inc., at Peoria, Illinois, with Anderson expellers. The hydraulic and solvent meals were prepared in the soy bean plants of the Archer-Daniels-Midland Company at Toledo, Ohio, and Chicago, Illinois, respectively.

each rat was kept in a separate cage. The animals were weighed at approximately the same time every 7 days. The length of the feeding period in all growth experiments was 56 days. For convenience these experiments have been grouped into three separate series, viz., 1, 2 and 3.

In series 1 the experiments determined the nutritive value of the protein of the raw soy beans and the various samples of commercial soy bean oil meal by the ad libitum type of feeding. Although most of the rations were given a protein content of 18 per cent, a few were formulated at 10 per cent. Two lots of rats were fed a diet containing casein—one lot at each protein level of 10 and 18 per cent.

As seen from the results, table 2, the ground soy beans supported only slight growth when fed at an 18 per cent protein level and no growth at 10 per cent. The soy bean oil meals also produced better growth with 18 per cent protein than at 10 per cent. The high temperature expeller meal excelled the low temperature expeller meal and the ground soy beans A by a considerable margin in both rate and efficiency of gains regardless of the level of protein. At the 18 per cent level, the high temperature expeller meal excelled the medium temperature expeller meal. The medium and high temperature hydraulic meals were about equal in nutritive value. These values, however, were significantly higher than for the protein of the low temperature hydraulic meal. The feeding of the commercial solvent soy bean oil meal resulted in good growth and a nutritive value of its protein equal to any of the other meals except the high temperature expeller meal. The nutritive value obtained for this solvent meal was more than three times that of the ground raw soy beans C. Casein was found to be superior to all samples of soy beans and soy bean oil meal in rate and efficiency of gains at a protein level of 10 per cent. At the 18 per cent level, however, casein was no better than the proteins of the high temperature soy bean oil meal prepared by the expeller process.

The average daily food intake in table 2 shows that the failure of the raw soy beans and low temperature meals to promote good growth was not due to a lack of food consumption. The similarity of food intake for the first few days suggests that the low nutritive value of the proteins of the raw soy beans and low temperature meals was due to some deficiency.

TABLE 2

The nutritive value of the proteins of the raw soy bean and soy bean oil meals prepared at different temperatures, compared with casein at protein levels of 10 and 18 per cent. Food intake ad libitum. (Series 1)[1]

LOT	DIET					LEVEL OF PROTEIN	AVERAGE DAILY FOOD INTAKE			GAIN IN BODY WEIGHT		GROWTH PER GRAM OF PROTEIN EATEN
	Casein	Soy beans			Supplement		3 days after 1st day	7 days after 1st day	Feeding period of 56 days	Range	Average	
		Kind	Process	Temperature								
	per cent					*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1		Gr. bean A		Raw		10	5.0	4.5	3.9	—12–+3	—(4.5)	
2		Oil meal	Expeller	105°C.		10	5.8	5.0	6.3	17– 39	23.5	0.65
3		Oil meal	Expeller	150°C.	Soy bean oil	10	6.8	6.8	7.2	20– 65	48.0	1.19
19	8.4				Agar (2 per cent)	10	6.3	6.5	8.8	26– 98	73.5	1.49
41		Gr. bean A		Raw		18	7.0	5.3	5.3	21– 36	27.3	0.51
6		Oil meal	Expeller	105°C.		18	4.5	5.0	5.7	18– 41	33.5	0.58
15		Oil meal	Expeller	130°C.	Soy bean oil	18	6.3	7.3	9.1	80–136	105.0	1.15
7		Oil meal	Expeller	150°C.	Soy bean oil	18	4.8	5.0	8.7	116–135	126.3	1.45
26		Oil meal	Hydraulic	82°C.	Soy bean oil	18	5.3	5.3	5.4	45– 78	59.7	0.82
17		Oil meal	Hydraulic	105°C.	Soy bean oil	18	6.0	7.0	10.3	77–190	120.3	1.14
18		Oil meal	Hydraulic	121°C.	Soy bean oil	18	7.3	7.5	10.5	103–134	122.3	1.15
45		Gr. bean C		Raw		18	4.5	4.8	5.0	11– 34	19.0	0.37
32		Oil meal	Solvent	98°C.		18	4.0	5.5	8.0	93–101	98.5	1.22
8	18.2				Agar (2 per cent)	18	5.3	6.3	10.4	141–163	150.8	1.44

[1] Each lot consisted of four rats.

In the experiments in series 2, table 3, the equalized type of feeding was employed. Six male rats of approximately the same age and weight constituted each experimental lot. All rats included in these experiments received the same total amount of food for the feeding period. This was determined by the intake of the rat which consumed the least food ad libitum, all lots considered. Adjustments were made in the daily portion of food weighed out so that the total food intake of each rat was the same by the end of each 7 days. The important advantage of this method over the paired feeding

TABLE 3

The nutritive value of the proteins of soy bean oil meals prepared at different temperatures. Equalized intake. (Series 2)[1]

LOT	DIET				GAIN IN BODY WEIGHT (56 DAYS)		GROWTH PER GRAM OF PROTEIN EATEN
	Soy beans			Supplement	Range	Average	
	Kind	Process	Temperature				
					gm.	gm.	gm.
33	Ground bean A		Raw		31–45	37.2	0.75
40	Oil meal	Expeller	105°C.		34–41	36.2	0.73
34	Oil meal	Expeller	150°C.	Soy bean oil	42–58	52.0	1.04
39	Ground bean B		Raw		35–46	39.0	0.78
36	Oil meal	Hydraulic	105°C.	Soy bean oil	45–60	53.0	1.06
35	Oil meal	Hydraulic	121°C.	Soy bean oil	46–63	54.8	1.10

[1] The level of protein for all diets was 18 per cent. The average daily food intake was 4.9 gm. Each lot consisted of six rats.

technic of Mitchell and Smuts ('32) is that it permits comparison of growth data obtained from one lot of animals whether treated individually or collectively with that of the animals of any lot within the same series. The constituents which formed the experimental diet for each lot in this series of experiments are given in table 3. The level of protein chosen for the diets was 18 per cent. The samples of ground raw soy beans A and B and the low temperature expeller soy bean oil meal were found to be equally inferior as a source of protein compared with the high temperature expeller meal and the medium and high temperature hydraulic meals.

In these growth experiments series 1 and 2 we found for the expeller and hydraulic soy bean oil meals a certain agreement between the color of the meals and the relative efficiency of the proteins. The inefficient low temperature expeller meal was light colored while the highly efficient medium and high temperature expeller meals were light brown and brown in color respectively. The less efficient low temperature hydraulic meal was also light colored while the more efficient medium and high temperature hydraulic meals were light brown and brown in color. In direct contrast with the expeller and hydraulic meals, the solvent meal, which contained an efficient protein, was light in color. These results suggest that brown color can only be used as an index of the probable efficiency of the proteins of commercial soy bean oil meals with the expeller and hydraulic meals.

The experiments of series 3 represent an initial attempt to determine the cause of the nutritive failure with raw soy beans. In some of our early experiments where the raw soy bean supplied the protein, excepting that contained in the yeast, the rats not only failed to make consistent growth but often showed a depraved appetite and unthrifty appearance usually leading to death in the sixth to the tenth week. The diets for each of the five lots in this series of experiments are listed with the results in table 4. The addition of casein to the ground soy bean diet resulted in good growth and a condition of well being. Due to the fact that the protein was fed at a high level its full nutritive value was not revealed. The results suggest that the cause for the failure of the ground raw soy bean to support appreciable growth in the rat in series 1 and 2 was directly due to a deficiency in good protein.

Removing the oil from the soy bean at room temperature did not result in any increase in the rate or efficiency of growth over that obtained in previous experiments where the ground raw soy beans were fed.

In lots where the ground whole soy bean, which had received various heat treatments, was included in the experimental diets, varying results were obtained. Dry heat for

1½ hours did not improve its nutritive value. This heated soy bean material had a color index similar to that of the high temperature expeller meal. Heating the ground soy bean in a sealed bomb for 1½ hours improved the nutritive value of the protein over that resulting from dry heating. In this process the original moisture of the ground bean was

TABLE 4

The growth promoting properties of soy beans as affected by additions of casein, by removal of the oil, and by various heat treatments. (Series 3)[1]

LOT	DIET			LEVEL OF PROTEIN	AVERAGE DAILY FOOD INTAKE			GAIN IN BODY WEIGHT		GROWTH PER GRAM OF PROTEIN EATEN
	Casein	Ground soy beans A			3 days after 1st day	7 days after 1st day	Feeding period of 56 days	Range	Average	
		Process	Temperature							
	per cent			*per cent*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
20	18		Raw	32	5.0	5.5	8.8	136–156	146.5	0.93
42		Heated in electric oven	135°C. 1½ hours	18	6.4	5.3	4.7	15– 30	22.3	0.48
50		Heated in sealed bomb in autoclave	17 lb. (125°C.) 1½ hours	18	6.3	6.8	8.9	45–116	83.3	0.93
51		Autoclaved	17 lb. (125°C.) 1½ hours	18	5.9	6.8	8.8	85–120	108.3	1.22
46		Oil extracted with ether	Room temperature 26°C.	18	6.2	6.6	5.5	9– 27	17.5	0.31

[1] Each lot consisted of four rats.

well preserved. The material was light brown in color. The most effective laboratory process for improving the nutritive value of the proteins of the ground whole soy bean proved to be that of autoclaving the ground material for 1½ hours with steam at 17 pounds pressure. This material was brown in color similar to that of the high temperature hydraulic soy bean oil meal.

Nitrogen balance experiments. These studies consisted of three nitrogen balance experiments designated respectively series 4, 5 and 6. Eight male rats of an average weight of about 85 gm. were used in each series. Series 4 and 5 consisted of two feeding periods each, while series 6 was made up of four periods, of which the first and fourth were used for feeding a nitrogen low ration. The periods were 10 days or more in length. The collection of excreta was made during the last 7 days of each period. The equalized intake type of feeding was employed in all. In each series the rats were divided into two lots of four rats each with the exception of the nitrogen free periods 1 and 4 of series 6. During the same period one lot was fed the raw soy bean diet and the other the heated soy beans. In the period following, the order of food assignment was reversed.

For the nitrogen balance experiments ground raw soy beans A and the high temperature expeller soy bean oil meal, processed at 140 to 150°C. for 2½ minutes, were fed in a basal ration in sufficient amounts to supply 18 per cent of protein. This basal ration was composed of about 45 parts cooked starch, 4 parts salts no. 40 (Steenbock and Nelson, '23) and 2 parts of cod liver oil. Soy bean oil was added in sufficient quantities to the diet containing soy bean oil meal to equal that supplied by the soy bean. The low nitrogen ration fed in periods 1 and 4, series 6, consisted of 15 parts butter fat, 8.4 parts soy bean oil, 2 parts cod liver oil, 4 parts salts no. 40, 4 parts agar, and 66.6 parts cooked starch. Vitamin B was supplied in the low nitrogen periods by giving each rat daily 40 mg. of an extract obtained from Parke, Davis and Company. This dosage of vitamin B extract contained 0.34 mg. of nitrogen.

The daily allowances of the experimental diets were weighed out on a sensitive balance. During the collection period the feces of each rat was transferred daily from the collector to small weighed flasks. These flasks contained a measured amount, usually 15 cc. of 2 per cent H_2SO_4. The urine collecting flasks contained about 10 cc. of a 2 per cent solution

of H_2SO_4. These flasks were emptied daily into large flasks which were of sufficient size to hold the 7-day collections plus washings. The surface of all glass-ware and other equipment exposed to the urine was washed daily with a warm 2 per cent solution of H_2SO_4 and distilled water. A double thickness of toweling was used to filter out any hairs or feed particles.

At the end of the collection period the feces were dried to constant weight at 100°C. The samples of feces were then ground to a uniform fineness in a steel mortar with a steel pestle and analyzed for nitrogen. The total urine collections were made up to a suitable volume from which an aliquot was analyzed for nitrogen.

The data for the low nitrogen periods are not presented in detail because the values obtained for the different rats were essentially similar. For example, the fecal nitrogen ranged from 5 to 7 mg. daily, the urinary nitrogen from 11 to 18 mg., the fecal nitrogen per gram of food eaten 1.4 to 1.9 mg., and the nitrogen in the urine per 100 gm. of body weight 16 to 20 mg. These values were for rats nos. 256 to 263, with the exception of rat 262 which was withdrawn from the experiment because it consumed some of its hair.

To conserve space the results of series 4 and 5 are not presented in detail. Briefly, in series 4 the raw and heated soy beans fed at 2.77 and 2.85 per cent of nitrogen gave apparent coefficients of digestibility of 77 and 85 per cent, respectively, with apparent biological values of 9 and 26. In this statement the adjective 'apparent' is used to designate the fact that the values have not been corrected for endogenous nitrogen according to the procedure of Mitchell and Villegas ('23) and Mitchell ('24). In series 5 at 2.85 and 2.95 per cent of nitrogen the respective values for heated and unheated beans were 80 and 83 for digestibility and 6 and 18 for apparent biological value.

The data of series 6 are presented in detail in table 5. However, only the corrected values are tabulated. It should be stated for purposes of comparison with series 4 and 5 that the apparent values with the raw and heated soy beans were,

TABLE 5

Nitrogen balances showing digestibility and biological values of raw and heated soy beans. (Series 6)

PERIOD	RAT NO.	BODY WEIGHT	DAILY FOOD INTAKE	DAILY FECAL N	DAILY URINE N	DAILY ENDOGENOUS N		DAILY FOOD N ABSORBED	DAILY FOOD N RETAINED	DIGESTION COEFFICIENT CORRECTED	BIOLOGICAL VALUE CORRECTED
						Fecal	Urine				
Raw soy bean ration 11 (2.89 per cent N)—daily N intake per rat = 145 mg.											
		gm.	*gm.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*per cent*	*per cent*
Second	256	82	5	31	90	10	16	124	50	85	40
Second	257	82	5	28	86	8	18	125	57	86	46
Second	258	85	5	34	78	7	15	118	55	81	47
Second	259	78	5	30	82	8	13	123	54	85	44
Third	260	92	5	30	99	10	16	125	42	86	34
Third	261	92	5	24	95	7	16	128	49	88	38
Third	263	96	5	30	88	8	15	123	50	85	41
									Average	85	41
Heated soy bean ration 13 (2.87 per cent N)—daily N intake per rat = 144 mg.											
Third	256	90	5	29	84	10	18	125	59	87	47
Third	257	90	5	30	82	8	19	122	59	85	48
Third	258	96	5	27	78	7	18	124	64	86	52
Third	259	90	5	25	72	8	15	127	70	88	55
Second	260	84	5	26	83	10	16	128	61	89	48
Second	261	84	5	27	76	7	15	124	63	86	51
Second	263	89	5	25	72	8	14	127	69	88	54
									Average	87	51

respectively, 80 and 81 for digestibility and 23 and 33 for biological value.

It is clearly shown that heating the raw soy bean caused an increase in the utilization of the absorbed nitrogen (biological value). The apparent biological values obtained in these experiments for the proteins of the soy bean are in agreement with the corrected values obtained in series 6. This harmony of results existing between uncorrected and corrected values for percentage of nitrogen retention is largely credited to both the short time of confinement for the rat of each series and the reversal plan of feeding.

The fact that heating increased the biological value of the protein helps to explain the increased gain per gram of protein which resulted from similar heat treatment in our growth experiments. While it is possible that the increase in efficiency of the protein resulted from the small increase in digestibility, it is not excluded that heat may have made available for metabolic use a protein fraction which was absorbed but not metabolically available. It remains to be seen if this can be established by metabolism trials for certain complexes as for example those which contain cystine. That cystine supplements raw soy bean proteins is well known (Mitchell and Smuts, '32; Shrewsbury and Bratzler, '33).

SUMMARY

Raw soy beans were found to contain protein of low nutritive value as determined by the grams of growth per gram of protein eaten. Commercial soy bean oil meals such as the expeller meal processed at low temperatures, 105°C. for 2 minutes or the hydraulic meal cooked at 82°C. for 90 minutes contained proteins similar in nutritive value to the raw soy beans. On the other hand, commercial soy bean oil meals which had been prepared at medium and high temperatures such as expeller meals processed at 112 to 130 and 140 to 150°C. for 2½ minutes or hydraulic meals cooked at 105 and 121°C. for 90 minutes contained proteins which had about twice the nutritive value of the raw soy beans or low temperature meals. These expeller and hydraulic meals prepared at

medium temperatures, respectively, were light brown in color while the meals prepared at high temperatures were brown in color. Heating the extracted soy beans at 98°C. for 15 minutes, as in the commercial solvent method of oil extraction, was also found to be an effective method of heat treatment. This solvent meal, however, was light colored. When the ground whole soy bean was autoclaved in the laboratory until the meal was brown in color, the protein had a high nutritive value. These results together with the fact that the commercial solvent meal was found to contain a very efficient protein suggest that brown color can only be used as an index of the probable efficiency of the proteins of commercial soy bean oil meals produced by the expeller and hydraulic processes.

The food intake of all rats which received either the raw or heated soy bean diets ad libitum was found to be similar for the first few days of the feeding period. This suggested that the poor growth resulting from the raw soy beans and low temperature meals was due to some deficiency in these constituents rather than to a lack of palatability.

When casein was incorporated in the diet which contained ground raw soy beans, normal growth resulted. These results suggested that the deficiency in the soy bean existed in the protein fraction.

Heating the raw soy bean to a high temperature in the expeller method of oil extraction caused an increase in the digestibility and biological value of the protein. The digestibility increased only about 3 per cent while the increase in biological value was about 12 per cent. This increase in biological or nutritive value of the protein established by metabolism tests agreed with the increase in nutritive value as determined by growth experiments.

The possibility appeared that heat caused some essential protein fraction, which was unavailable in the raw soy bean, to become available for absorption and metabolic use.

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METABOLISM OF WOMEN DURING THE REPRODUCTIVE CYCLE

VII. UTILIZATION OF INORGANIC ELEMENTS (A CONTINUOUS CASE STUDY OF A MULTIPARA)[1]

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THREE FIGURES

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As the studies on the metabolism of women during the reproductive cycle have progressed in this laboratory over a period of years, it has become increasingly apparent that—

it is expedient to extend, not only the short balance to a longer period of time, but to determine uninterruptedly the metabolic response of the same mother during the development of the fetus and the physiological preparation of her own body for lactation, and thereafter, to extend these observations into parturition, puerperium and lactation, thereby learning more specifically where the stresses and stains of child-bearing and child-rearing lay (Hunscher, Hummel, Erickson and Macy, '35).

Heretofore, nearly all the emphasis has been laid on a few of the more obvious inorganic elements in nutrition especially those that are known to be intimately related to protein, blood and bone formation to the exclusion of other similarly essential nutrients. Such substances may be needed in the body in only small quantities and even in traces, but nevertheless, they play important metabolic functions in the body

[1] The subject of this investigation has been referred to in other publications from this laboratory as L.R. and subject VII.

and may influence greatly through their chemical affinity or antagonism the utilizability of other substances in the body.

To date the quantitative studies[2] in pregnancy have included, for the most part, intermittent metabolic balances on calcium, magnesium, phosphorus with but one report each on iron, sulfur and acid-base mineral with the exception of Hoffström ('10) who observed continuously calcium, magnesium and phosphorus retentions and sulfur outgo on one woman from the seventeenth week of pregnancy to term. The only studies on inorganic balances in lactation and post lactation have been those reported by Toverud and Toverud ('31); Bauer, Albright and Aub ('29), and this laboratory (Hunscher, '30; Macy, Hunscher, McCosh and Nims, '30; Donelson, Nims, Hunscher and Macy, '31). Heretofore, no studies have been made continuously during the entire reproductive cycle including pregnancy, puerperium and lactation. The present paper records data pertaining to the progressive utilization or loss and the continuous chemical exchange of calcium, magnesium, sodium, potassium, sulfur, phosphorus and chlorine by a multipara during 21 weeks of pregnancy, and after 10 days' puerperium to the eighth week postpartum, an observation period approximating 8 months; for comparative purposes data collected from balances made at intervals during the two preceding reproductive cycles are included.

EXPERIMENTAL

Subject. An American woman (L.R.)[1] who had participated over a 5-year period in many types of nutritional and physiological studies relating to the metabolism of the reproductive cycle, including the chemistry of milk and factors affecting milk secretion, offered her services again for this long and intensive metabolic study during the early part of her fourth reproductive cycle, of which the data are recorded herein and in a previous publication (Hunscher, Hummel, Erickson and Macy, '35). Frequent medical examinations

[2] An inclusive bibliography on mineral utilization in pregnancy has not been compiled since several comprehensive reviews have appeared recently.

not only during the second and third reproductive cycles but during the present or fourth verified the absence of metabolic disturbances, and the usual dyscrasias of conception; for instance, there was no nausea, no gastro-intestinal disturbances, no albuminuria, no unusual changes in body weight during either pregnancy or lactation.[3] While under the observations of the staff of this laboratory during her second and third reproductive cycles she had successfully borne nutritionally stable children who have continued to develop both physically and mentally in a most satisfying manner without known metabolic disturbances as well as few infections and diseases of childhood. During her three initial lactation periods and the present one she secreted considerable quantities of breast milk beyond what her babies needed because she gave herself a maximum stimulus of complete and frequent removal of all the milk from the mammae (Macy, Hunscher, Donelson and Nims, '30). The quantity of milk was no greater perhaps than the average mother would produce who successfully bears and breast feeds twins nor any other healthy robust woman who has the inherent ability to secrete milk were the same stimulus persistently applied and were analagous hygiene and sanity of living rigidly practiced. After many years of careful study of the physiological functions of child-bearing and child-rearing we have grown to consider this woman approaching the optimal because she has so successfully carried out all the functions of motherhood in an average sized family and, in addition, maintained her own self in an admittedly superior state of health. Certainly with our present medical and nutritional knowledge of the physiology of maternity it would be difficult to offer any specific suggestions for improvement of either the physical well-being of this woman or her offspring.

Dietary. The diet was voluntarily chosen as to quantity and quality and it included 2 quarts of milk in each of which were incorporated 400 U.S.P. units of cod liver oil concentrate

[3] The authors are grateful to A. S. Guimaraes, M.D., for his interest and cooperation.

(Vitex).[4] The diet of her own free choice was abundant in all known nutritive essentials and in this food consumption there was an average of 3389 (2490 to 4324) calories and of 19 (16.8 to 22.33) gm. of nitrogen (Hunscher, Hummel, Erickson and Macy, '35).

General procedure. The subject remained in her own home performing her usual home duties except during delivery and the lying-in period.[5] Successive metabolic mineral balances of 5 days' duration were begun in the fourth reproductive cycle at approximately the one hundred and thirty-fifth day after conception and carried thereafter continuously through the first 2 months of lactation with only an interruption of the initial 10 days' postpartum. The metabolic collection procedures adhered to in this laboratory and described in a previous publication (Hunscher, Hummel, Erickson and Macy, '35) were followed.

Methods. The inorganic elements in the urine were determined according to the following procedures. Calcium and magnesium were determined by the McCrudden method ('09–'10; '11) with certain adaptations. The precipitated calcium oxalate was finally washed with cold water, dissolved in hot H_2SO_4 and titrated with 0.05 N potassium permanganate. On two occasions, however, when large amounts of urates were present in the urine the use of the method of Shohl and Pedley ('22) seemed advantageous. In all cases the filtrate and washings were used for the determination of magnesium as outlined in the McCrudden procedure. For the sodium and potassium analyses the combined sulfates were obtained and weighed according to the classic Lindo-Gladding ('24) method of the Association of Official Agricultural Chemists as adapted to biological materials by Mackay and Butler and given in Peters and Van Slyke ('32). Sodium was determined on a

[4] We wish to express our thanks and appreciation to Bion East, D.D.S., of the Vitex Laboratories, Inc., Harrison, N. J., for furnishing the fortified vitamin D milk for this study.

[5] It is a pleasure to acknowledge the helpful cooperation of the medical, nursing and dietetic staffs of the Florence Crittenton Hospital, Detroit, in the care of the patient during the 10 days' confinement period.

fresh sample of urine by the uranyl acetate micro gravimetric method of Barber and Kolthoff as modified by Butler and Tuthill ('31). The amount of sodium sulfate was then calculated and the potassium obtained by difference. The other inorganic elements were determined by the following procedures: chlorine by the Volhard-Arnold titration method described in Hawk and Bergeim, '31; phosphorus by the colorimetric micro method of Youngburg and Youngburg, '30; and sulfur by the Benedict ('09) gravimetric method for total sulfur in urine.

In the case of food and feces the above methods were used for sodium, chlorine, phosphorus and sulfur but for the other elements certain changes seemed advantageous in our hands. In determining calcium the micro titration method by Hawks ('31) was used with samples of 2.0 to 2.5 gm. of food and approximately 1.75 gm. of feces. For magnesium, the Greenberg and Mackey method ('32) was adapted with the following modifications: the supernatant fluid and washings from the calcium precipitation were evaporated to dryness on the hot plate with 5 cc. of HNO_3. The residue was dissolved in 25 cc. of water to which 1 cc. of 2 per cent NH_4Cl, 2 cc. of 2 per cent 8-hydroxyquinoline (in 95 per cent alcohol), 20 drops of NH_4OH were added and the whole thoroughly mixed. The solution was then heated on a steam bath for 25 minutes, transferred to filter with three minimal portions of 2 per cent NH_4OH, and finally washed six times with water. The precipitate was dissolved in 20 cc. hot 1:4 HCl and the filter washed six times with water. One cubic centimeter of saturated KBr and 5 cc. of M/30 $KBrO_3$ were added, the tube was then stoppered, shaken and let stand for 30 to 60 seconds. One cubic centimeter of 20 per cent KI was added and the solution titrated with 0.05 N $Na_2S_2O_3$ to a starch endpoint. The cobaltinitrite micro titration method of Clausen ('18), and Kramer and Tisdall ('21), as outlined by Peters and Van Slyke ('32) was used for potassium.

RESULTS AND DISCUSSION

Tables 1 to 3 give a summary of the metabolic data accumulated on the continuous balances of the base-forming inorganic elements, calcium, magnesium, sodium, potassium and the acid-forming inorganic elements of phosphorus, sulfur and chlorine of one woman (L.R.) in her fourth reproductive cycle. The quantities of these inorganic elements taken into the body over a period of approximately 8 months beginning with the final 145 days of gestation and from the tenth to fifty-third days postpartum including the period of heaviest milk flow (Macy, Hunscher, Donelson and Nims, '30), the paths of excretion of the same elements in the urine, feces and breast milk, together with maternal gain or loss are recorded. In addition, the present data are enhanced in value through their supplementation by results from individual short time calcium balance periods at intervals in the second lactation and during the third cycle including post lactation as shown in figure 1 (Hunscher, '30; Macy, Hunscher, McCosh and Nims, '30; Donelson, Nims, Hunscher and Macy, '31).

Pregnancy. The results of the calcium, magnesium, sodium and potassium balances are given in table 1 and of phosphorus, sulfur and chlorine in table 2. For the most part in gestation positive retentions predominated in the case of all the observed inorganic elements but there were noticeable fluctuations from period to period and even losses in some instances. With the mean daily intake of 3.09 ± 0.15, 0.60 ± 0.02, 5.05 ± 0.51 and 6.60 ± 0.56 gm. of calcium, magnesium, sodium and potassium, the mean maternal retentions were 0.37 ± 0.19, 0.11 ± 0.05, 0.56 ± 0.53 and 1.40 ± 0.72 gm., respectively, and mean intake of 2.67 ± 0.22, 1.50 ± 0.15 and 7.68 ± 0.96 of phosphorus, sulfur and chlorine the mean retentions were 0.26 ± 0.16, 0.34 ± 0.13 and 0.60 ± 0.96 gm., respectively. From these data it will be noted from table 4 that the mean daily balances for calcium, magnesium, potassium and sulfur were greater in the present uninterrupted study in pregnancy on one woman than those obtained in previous observations in which isolated periodic balances

TABLE 1

Inorganic acid-forming elements retained per day by a woman (L.E.) who was studied uninterruptedly throughout the last half of pregnancy in her fourth reproductive cycle

DAY OF GESTATION	CALCIUM				MAGNESIUM				SODIUM				POTASSIUM			
	Intake	Urine	Feces	Balance	Intake	Urine	Feces	Balance	Intake	Urine	Feces	Balance	Intake	Urine	Feces	Balance
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
135–141	2.86	0.43	2.18	0.25	0.56	0.15	0.38	0.03	4.99	4.35	0.05	0.59	6.07	3.48	0.73	1.86
142–148	3.05	0.45	2.44	0.16	0.66	0.17	0.44	0.05	5.43	4.62	0.05	0.76	6.38	3.54	0.79	2.05
149–155	3.07	0.44	2.28	0.35	0.53	0.17	0.33	0.03	4.92	4.27	0.08	0.57	5.88	3.76	0.69	1.43
156–162	2.96	0.42	2.28	0.26	0.64	0.16	0.41	0.07	4.26	4.16	0.06	0.04	7.14	4.76	0.82	1.56
163–167	2.94	0.44	2.22	0.28	0.58	0.16	0.39	0.03	5.46	4.52	0.09	0.85	6.88	4.70	0.67	1.51
168–170	2.90	0.36	2.02	0.52	0.59	0.11	0.42	0.06	4.36	3.31	0.09	0.96	6.72	4.65	0.95	1.12
171–175	3.11	0.36	2.32	0.43	0.71	0.14	0.43	0.14	5.34	3.44	0.07	1.83	7.58	4.90	0.87	1.81
176–180	3.15	0.42	2.15	0.58	0.56	0.07	0.33	0.16	6.01	5.75	0.08	0.18	6.44	4.66	0.59	1.19
181–185	3.06	0.50	2.25	0.31	0.61	0.16	0.39	0.06	5.28	4.96	0.13	0.19	6.33	4.21	0.68	1.44
186–190	2.89	0.38	1.97	0.54	0.48	0.08	0.30	0.10	2.87	2.99	0.21	— 0.33	5.38	3.67	0.73	0.98
191–195	3.17	0.33	2.48	0.36	0.76	0.10	0.45	0.21	5.13	3.78	0.09	1.26	7.26	3.25	0.86	3.15
196–200	3.08	0.33	2.09	0.66	0.61	0.10	0.37	0.14	4.66	3.62	0.09	0.95	6.56	5.24	0.69	0.63
201–205	2.78	0.38	2.11	0.29	0.50	0.11	0.29	0.10	4.11	4.06	0.10	— 0.05	5.77	4.71	0.59	0.47
206–210	3.04	0.41	2.44	0.19	0.65	0.11	0.43	0.11	5.57	4.95	0.11	0.51	7.25	3.60	0.69	2.96
211–215	3.18	0.42	2.50	0.26	0.55	0.11	0.36	0.08	5.18	4.97	0.12	0.08	7.05	4.87	0.71	1.47
216–220	3.00	0.39	2.28	0.33	0.52	0.14	0.31	0.07	5.59	4.55	0.08	0.96	6.42	4.30	0.63	1.49
221–225	3.17	0.43	2.65	0.09	0.66	0.11	0.45	0.10	6.24	4.96	0.09	1.19	7.11	4.51	0.70	1.90
226–230	3.35	0.40	2.35	0.60	0.62	0.12	0.52	0.10	5.13	4.94	0.17	0.02	7.49	5.21	0.71	1.57
231–235	3.08	0.46	2.30	0.32	0.58	0.13	0.32	0.13	4.51	4.37	0.08	0.06	6.91	5.09	0.57	0.25
236–240	3.16	0.44	2.33	0.39	0.57	0.09	0.34	0.14	5.14	5.09	0.13	— 0.08	6.77	5.01	0.73	1.03
241–245	2.97	0.44	2.36	0.17	0.62	0.15	0.38	0.09	5.28	4.95	0.08	0.25	7.19	5.36	0.59	1.24
246–250	3.14	0.49	2.50	0.15	0.72	0.11	0.38	0.23	5.21	3.83	0.10	1.28	7.25	5.35	0.79	1.11
251–255	3.28	0.41	2.36	0.51	0.57	0.17	0.31	0.09	4.41	3.07	0.11	1.23	5.93	5.76	0.53	— 0.36
256–260	3.11	0.51	1.99	0.61	0.58	0.16	0.31	0.11	5.03	4.54	0.07	0.42	6.01	3.13	0.58	2.30
261–265	3.25	0.50	2.22	0.53	0.59	0.10	0.32	0.17	4.97	4.46	0.08	0.43	6.11	4.42	0.59	1.10
266–270	3.16	0.39	2.58	0.19	0.60	0.11	0.40	0.09	4.45	3.24	0.07	1.14	6.14	3.98	0.52	1.64
271–275	3.33	0.43	1.99	0.91	0.62	0.13	0.29	0.20	4.31	3.62	0.03	0.66	6.26	4.49	0.40	1.37
276–280	3.29	0.46	2.69	*0.14*	0.62	0.17	0.39	*0.06*	4.99	4.88	0.26	*— 0.15*	6.57	4.91	0.81	*0.85*
Total gain—145 days (final)				*52.90*				*15.48*				*81.00*				*207.16*
Mean	*3.09*			*0.37*	*0.60*			*0.11*	*5.05*			*0.56*	*6.60*			*1.40*

TABLE 2

Inorganic acid-forming elements retained per day by a women (L.R.) who was studied uninterruptedly throughout the last half of pregnancy in her fourth reproductive cycle

DAY OF GESTATION	PHOSPHORUS				SULFUR				CHLORINE			
	Intake	Urine	Feces	Balance	Intake	Urine	Feces	Balance	Intake	Urine	Feces	Balance
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
135–141	2.64	1.36	1.08	0.20	1.62	0.96	0.17	0.49	7.32	6.73	0.09	0.50
142–148	2.85	1.29	1.23	0.33	1.57	1.05	0.23	0.29	8.05	6.68	0.10	1.27
149–155	2.68	1.51	1.06	0.11	1.38	0.97	0.18	0.23	7.56	6.42	0.12	1.03
156–162	2.67	1.20	1.17	0.30	1.72	0.96	0.18	0.58	7.86	7.05	0.11	0.70
163–167	2.70	1.26	1.16	0.28	1.51	1.01	0.18	0.32	8.42	7.47	0.11	0.84
168–170	2.49	1.00	1.13	0.36	1.39	0.97	0.16	0.26	5.95	6.08	0.09	—0.18
171–175	3.08	1.29	1.13	0.66	1.86	1.07	0.23	0.56	7.93	5.75	0.11	2.07
176–180	2.58	1.25	0.91	0.42	1.43	0.96	0.15	0.32	9.17	9.12	0.08	—0.03
181–185	2.61	1.61	0.95	0.05	1.55	1.13	0.20	0.22	7.81	7.71	0.12	—0.02
186–190	2.24	1.07	0.88	0.29	1.13	0.82	0.15	0.16	4.65	7.08	0.10	—2.53
191–195	3.08	1.39	1.34	0.35	1.69	0.95	0.23	0.51	7.49	5.72	0.09	1.68
196–200	2.73	1.14	1.03	0.56	1.47	0.92	0.16	0.39	7.06	5.59	0.09	1.38
201–205	2.41	1.19	0.97	0.25	1.40	0.84	0.19	0.37	6.42	6.30	0.10	0.02
206–210	2.85	1.47	1.14	0.24	1.52	1.01	0.19	0.52	8.49	7.50	0.11	0.88
211–215	2.77	1.59	1.10	0.08	1.71	1.03	0.19	0.49	8.28	8.11	0.12	0.05
216–220	2.67	1.56	1.06	0.05	1.49	1.10	0.15	0.24	8.55	6.99	0.09	1.47
221–225	2.81	1.54	1.15	0.12	1.58	1.04	0.18	0.36	9.35	7.54	0.09	1.72
226–230	2.72	1.32	0.91	0.49	1.53	1.02	0.17	0.34	8.16	7.62	0.14	0.40
231–235	2.69	1.54	0.85	0.30	1.62	0.99	0.14	0.49	7.26	7.16	0.09	0.01
236–240	2.61	1.40	0.98	0.23	1.44	1.06	0.16	0.22	8.31	8.09	0.15	0.07
241–245	2.61	1.41	0.98	0.22	1.71	0.98	0.18	0.55	8.50	7.89	0.08	0.52
246–250	2.76	1.56	1.08	0.12	1.45	0.98	0.20	0.27	8.44	6.27	0.11	2.06
251–255	2.51	1.52	0.90	0.09	1.23	0.96	0.15	0.12	6.97	5.10	0.10	1.77
256–260	2.68	1.49	0.99	0.20	1.47	1.00	0.16	0.31	7.82	7.59	0.08	0.15
261–265	2.66	1.69	0.82	0.15	1.40	0.90	0.17	0.33	7.67	7.16	0.11	0.40
266–270	2.43	1.52	0.96	—0.05	1.28	0.84	0.19	0.25	7.07	5.81	0.09	1.17
271–275	2.70	1.50	0.70	0.50	1.48	0.98	0.13	0.37	6.62	6.33	0.06	0.22
276–280	2.71	1.40	1.02	*0.29*	1.47	0.94	0.23	*0.30*	7.73	8.41	0.22	*—0.90*
Total gain—final 145 days				*37.11*				*50.96*				*91.26*
Mean	*2.67*			*0.26*	*1.50*			*0.34*	*7.68*			*0.60*

TABLE 3

Inorganic base-forming and acid-forming elements retained per day by a woman (L.R.) who was studied uninterruptedly from the tenth to fifth-third day of lactation in her fourth reproductive cycle

DAYS POST-PARTUM	INTAKE	URINE	FECES	MILK	BALANCE	INTAKE	URINE	FECES	MILK	BALANCE
	Calcium					Magnesium				
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
10–17	3.00	0.45	2.63	0.84	— 0.92	0.56	0.10	0.42	0.15	— 0.11
18–22	3.24	0.19	2.51	0.53	+ 0.01	0.63	0.04	0.41	0.12	+ 0.06
23–27	3.15	0.28	3.93	0.61	— 1.67	0.65	0.07	0.87	0.11	— 0.40
28–32	3.22	0.12	3.00	0.60	— 0.50	0.63	0.10	0.42	0.11	0.00
33–37	3.09	0.06	2.23	0.65	+ 0.15	0.58	0.11	0.35	0.12	0.00
38–42	3.04	0.07	2.43	0.61	— 0.07	0.71	0.05	0.44	0.12	+ 0.10
43–47	3.53	0.06	3.00	0.72	— 0.25	0.72	0.10	0.51	0.12	— 0.01
48–53	3.19	0.07	3.18	0.52	— 0.58	0.86	0.15	0.53	0.09	+ 0.09
Total for 43 days of lactation					*— 20.99*					*— 1.57*
Mean	*3.18*				*— 0.48*	*0.67*				*— 0.03*
	Sodium					Potassium				
10–17	4.80	2.63	0.15	0.46	+ 1.56	6.19	3.96	0.71	1.43	+ 0.09
18–22	4.69	3.73	0.11	0.42	+ 0.43	6.33	3.93	0.61	1.10	+ 0.69
23–27	5.64	4.75	0.31	0.36	+ 0.22	7.20	4.51	1.12	1.06	+ 0.51
28–32	5.74	4.81	0.14	0.28	+ 0.51	7.34	4.76	0.68	1.52	+ 0.38
33–37	4.69	4.10	0.08	0.32	+ 0.19	5.83	4.40	0.51	1.19	— 0.27
38–42	5.86	5.12	0.09	0.27	+ 0.38	7.00	1.84	0.57	1.18	+ 3.41
43–47	5.82	4.79	0.10	0.31	+ 0.62	7.57	4.31	0.69	1.32	+ 1.25
48–53	5.21	4.89	0.17	0.23	— 0.08	8.31	5.69	0.74	1.01	+ 0.87
Total for 43 days of lactation					*+ 22.27*					*+ 34.83*
Mean	*5.31*				*+ 0.47*	*6.97*				*+ 0.87*
	Phosphorus					Sulfur				
10–17	2.59	1.58	1.14	0.42	— 0.55	1.30	0.99	0.18	0.05	+ 0.08
18–22	2.63	1.49	1.10	0.25	— 0.21	1.20	0.80	0.17	0.48	— 0.25
23–27	2.87	1.40	1.90	0.28	— 0.71	1.28	1.03	0.35	0.53	— 0.63
28–32	2.76	1.28	1.19	0.28	+ 0.01	1.25	0.75	0.20	0.45	— 0.15
33–37	2.61	1.83	0.97	0.33	— 0.52	1.29	0.82	0.14	0.54	— 0.21
38–42	2.91	1.73	1.10	0.35	— 0.27	1.55	0.89	0.18	0.67	— 0.19
43–47	2.96	1.91	1.24	0.40	— 0.59	1.59	0.92	0.20	0.81	— 0.24
48–53	3.13	1.64	1.30	0.30	— 0.10	1.67	1.04	0.21	0.57	— 0.15
Total for 43 days of lactation					*— 15.80*					*— 8.70*
Mean	*2.81*				*— 0.37*	*1.39*				*— 0.22*
	Chlorine									
10–17	7.83	4.51	0.20	0.87	+ 2.25					
18–22	7.19	5.65	0.13	0.87	+ 0.54					
23–27	8.66	7.70	0.22	0.81	— 0.07					
28–32	9.22	7.70	0.11	0.76	+ 0.65					
33–37	7.39	6.61	0.09	0.95	— 0.26					
38–42	8.63	7.54	0.10	0.75	+ 0.24					
43–47	8.65	7.58	0.09	0.95	+ 0.03					
48–53	7.47	7.55	0.18	0.71	— 0.97					
Total for 43 days of lactation					*+ 16.55*					
Mean	*8.13*				*+ 0.39*					

TABLE 4

Summary of inorganic base-forming and acid-forming elements retained per day by a multipara (L.R.) during pregnancy and lactation compared with data from the literature

	PREGNANCY								LACTATION			
	Intake				Balance				Intake, present study		Balance, present study	
	Present study		Macy-Hunscher,[1] mean	Coons et al.,[2] mean	Present study		Macy-Hunscher,[1] mean	Coons et al.,[2] mean				
	Mean	Standard deviation			Mean	Standard deviation			Mean	Standard deviation	Mean	Standard deviation
	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.	gm.
Calcium	3.09	0.15	1.44	1.45	0.37	0.19	0.17	0.28	3.18	0.15	— 0.48	0.56
Magnesium	0.60	0.02	0.34	0.39	0.11	0.05	0.06	0.06	0.67	0.03	— 0.03	0.15
Sodium	5.05	0.51		4.91	0.56	0.53		1.26	5.31	0.49	+ 0.47	0.46
Potassium	6.60	0.56		3.64	1.40	0.72		0.51	6.97	0.80	+ 0.87	1.06
Phosphorus	2.67	0.22	1.91	1.64	0.26	0.16	0.31	0.30	2.81	0.18	— 0.37	0.24
Sulfur	1.50	0.15		0.76	0.34	0.13	0.05	0.004	1.39	0.17	— 0.22	0.18
Chlorine	7.68	0.96		5.82	0.60	0.96		0.89	8.13	0.70	+ 0.39	0.88
Base (excess)[3]	1221	164		1037	347	246		436	1350	292	471	423

[1] Macy, I. G., and H. A. Hunscher, '34.

[2] Coons, Coons and Schiefelbusch, '34.

[3] Amount of base in excess of acid (0.1 N).

were the source of information on these minerals (Macy and Hunscher, '34; Coons, Coons and Schiefelbusch, '34). From the continuous metabolic balances in the present study there

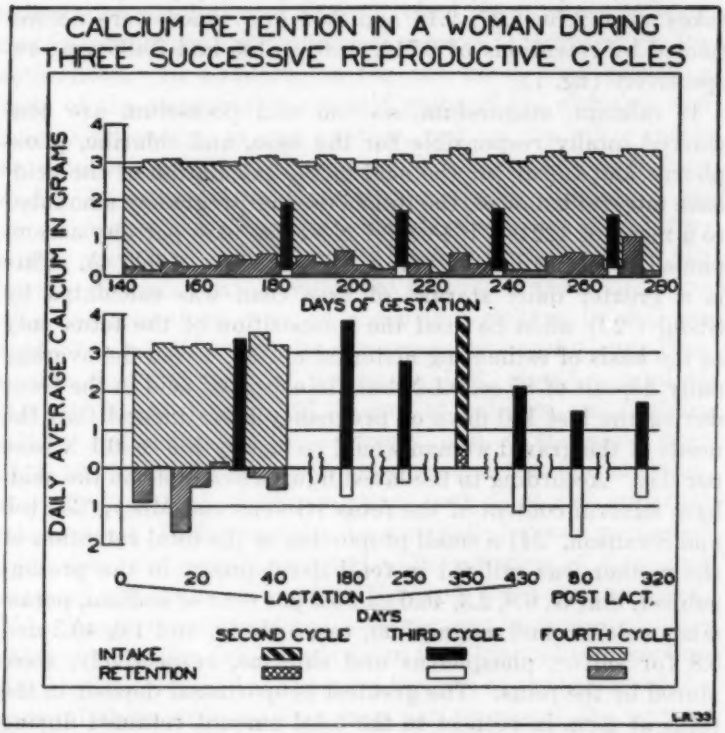

Fig. 1 Shows the continuous large calcium storage by a multipara during the last 145 days of pregnancy and from the tenth to fifty-third day of lactation in her fourth reproductive cycle when she was taking a diet fortified with a generous supply of minerals and vitamin D. Superimposed upon this curve are isolated 5- and 10-day balances that were made during two preceding cycles.

was no relationship of the amount of the elements retained to the progressive needs of gestation nor to the fluctuations of the intake. In the third reproductive cycle of the present subject at the twenty-sixth, thirtieth, thirty-fourth and thirty-

eighth weeks 4-day metabolic balances were conducted. The average daily calcium intakes were 1.92, 1.71, 1.76 and 1.60 gm. and the resultant retentions were 0.185, 0.278, 0.163 and 0.234 gm., respectively, and the average daily phosphorus intakes were 2.75, 2.27, 2.16 and 2.04 gm. which were accompanied by retentions of 0.712, 0.064, 0.135 and 0.468 gm., respectively (fig. 1).

If calcium, magnesium, sodium and potassium are considered totally responsible for the base, and chlorine, phosphorus and sulfur for the acid in the calculation of the acid-base mineral balances, the intake during pregnancy amounted to a mean of 1221 $\pm$ 164 cc. 0.1 N excess base per day accompanied by a mean retention of 347 $\pm$ 246 cc. (table 4). This is a greater daily storage of base than was calculated by Shohl ('23) when he used the composition of the fetus only as the basis of estimating maternal needs. From the average daily deposit of 85 cc. 0.1 N base in excess of acid in the fetus during the last 100 days of pregnancy he estimated that the needs of the gravid woman would be met by 150 cc. 0.1 N base per day. According to the latest figures available on the acid-base mineral content of the fetus (Givens and Macy, '33; Iob and Swanson, '34) a small proportion of the total retention of the mother was utilized in fetal development in the present subject, that is, 6.8, 2.3, 46.0 and 5.2 per cent of sodium, potassium, calcium and magnesium, respectively, and 1.6, 40.3 and 5.8 for sulfur, phosphorus and chlorine, respectively, were stored by the fetus. The greatest proportional deposit in the fetus at term in respect to the total amount retained during the latter half of pregnancy was in calcium and phosphorus; these elements were also shown to predominate in fetal chemical composition (fig. 2). Our results on acid-base mineral retention are comparable to those of Coons and co-workers (Coons, Coons and Schiefelbusch, '34; Coons, Schiefelbusch, Marshall and Coons, '35) who have recently reported a study on the acid-base mineral metabolism on five women at intervals during gestation (twenty acid-base balances) in which they showed an average storage of 436 $\pm$ 28 cc. 0.1 N base

in excess of acid daily. Only one of their twenty balances resulted in a retention less than Shohl's calculated requirement.

The uninterrupted balances in pregnancy show the total maternal accumulation of the individual elements which were used in fetal growth and maternal reserve in preparation for motherhood. In addition, on the one hand, they permit a study of the comparative rates of gains of the elements and their relationships to one another, and on the other hand, the detection of rates of changes in progressive gestatory needs. In contrast the short time metabolic studies, at intervals during the advancing reproductive cycle, give information upon food utilization at a specific time and under the existing physiological activities but because of the wide fluctuations of retentions even on a constant dietary intake, too rigid interpretations may lead to erroneous conclusions. During the final 145 days of pregnancy L.R. stored a total of 52.90, 15.48, 81.00 and 207.16 gm. of calcium, magnesium, sodium and potassium, respectively, and at the same time 37.11, 50.96 and 91.26 gm. of phosphorus, sulfur and chlorine, respectively, all of which constitute both fetal and maternal enhancement. Unfortunately, the metabolic balance method of study does not permit an exact differentiation of fetal and maternal utilization (Hunscher, Hummel, Erickson and Macy, '35). Rough approximations, however, can be made by calculating progressive fetal needs from the chemical composition of specimens obtained at autopsy; because of the nature of the material these data may or may not represent the normal mineral utilization in utero by the fetus. For example, by application of this method of study, of the 52.90 gm. of calcium laid down by L.R. from about the one hundred and thirty-fifth day to term, approximately 24 gm. went to the fetus leaving a known maternal reserve of about 29 gm. (fig. 2). Since this mother did not experience any of the usual dyscrasias following conception it is within reason to assume that the final maternal storage of pregnancy was even greater than recorded in this report since she, no doubt, was acquiring

considerable quantities of the various nutrients during the initial half of the period before these quantitative observations were begun. Similarly, the fetal requirements and the maternal reserves for the other six elements can be computed. The total storage of calcium, phosphorus and magnesium in our subject are comparable with those recorded by Hoffström ('10), the only other uninterrupted study that has been made in the last half of the gravid period.

The rates of gain of the seven inorganic elements and nitrogen in pregnancy are shown in figures 2 and 3. These curves were plotted on semi-logarithmic paper so as to illustrate the relative rates of gain of the different elements with the progression of the reproductive cycle and, in addition, shows their actual total accumulation during the final 145 days of gestation. It is of interest to note that the rate of accumulation of nitrogen, sulfur, potassium and sodium are similar; with the exception of sodium these elements predominate in the formation of soft tissue. Chlorine, after the one hundred and eightieth day in the period of pregnancy approximates the rate of gain in calcium and phosphorus, all of which are more rapidly accumulated than the above elements. Calcium and phosphorus are notable constituents in bone formation. In contrast, after the one hundred eightieth day the rate of magnesium storage was more rapid than the other seven elements which may be an assalient feature when considered in the light of the magnesium content of human embryos (Givens and Macy, '33). Together with supplemental studies[6, 7] of various types in progress in this laboratory these relationships will be given further detailed considerations. It can be stated, however, from data presented in the semi-logarithmic charts that in the last half of pregnancy there was no apparent change in the progressive gestatory needs as might have been inferred had isolated balances per-

[6] Continuous inorganic mineral balances on a young primipara is to be published shortly.

[7] An extensive study on the successive metabolic balances in childhood will be published in the near future.

chance been made at intervals when physiological and psychological conditions were such that made possible greater storage of the various nutrients and therefore, larger metabolic balances (fig. 1).

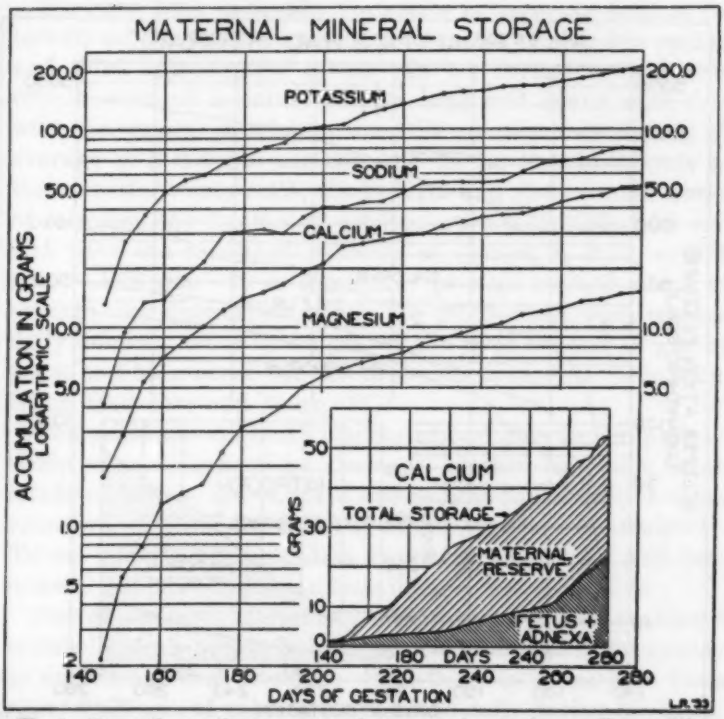

Fig. 2 Shows the continuous maternal storage of potassium, sodium, calcium and magnesium during the final 145 days of gestation. The days are plotted arithmetically and the maternal accumulation in grams logarithmically thus illustrating not only the absolute values but the relative rates of change with the progression of fetal development. The insert shows the large determined maternal reserve of calcium that is stored beyond the estimated needs of the fetus and its adnexa.

Lactation. In contrast to pregnancy, the physiological activity of lactation resulted in lowered retention and, in some cases, negative balances. Tables 3 and 4 show the data on

the continuous metabolic balances from the tenth to fifty-third day postpartum. The mean daily intakes and their standard deviations of the base-forming inorganic elements calcium, magnesium, sodium and potassium were 3.18 ± 0.15, 0.67 ±

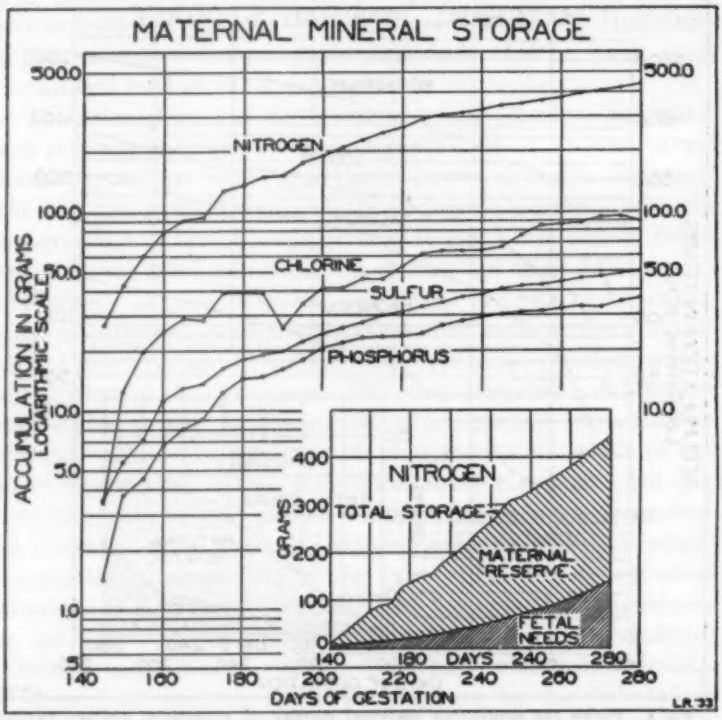

Fig. 3 Shows the continuous storage of nitrogen, chlorine, sulfur and phosphorus during the final 145 days of gestation. The days are plotted arithmetically and the maternal accumulation in grams logarithmically thus illustrating the absolute values and the relative rates of change with the progression of fetal development. The insert shows the large determined maternal storage of nitrogen that has been laid down by the mother in excess of the fetal needs.

0.03, 5.31 ± 0.49 and 6.97 ± 0.80 gm. which resulted in metabolic balances of — 0.48 ± 0.56, — 0.03 ± 0.15, + 0.47 ± 0.46, + 0.87 ± 1.06 gm., respectively, while the mean daily intakes

were 8.13 ± 0.70, 2.81 ± 0.18, 1.39 ± 0.17 gm. for the acid-forming chlorine, phosphorus and sulfur which were followed by balances of 0.39 ± 0.88, — 0.37 ± 0.24, — 0.22 ± 0.18 gm., respectively.

We have been unusually fortunate in securing data at intervals on the present subject in lactation during her second and third reproductive cycles. In the former cycle in the fiftieth week of lactation after a long and heavy milk flow when her intake of calcium was 3.92 gm. she was storing an average of 0.486 gm. per day but during the third cycle at the seventh, twenty-sixth, thirty-sixth and sixty-second weeks of lactation the intakes of calcium were 3.36, 3.83, 2.76 and 2.11 with the resultant balances of — 0.06, — 0.75, — 0.38 and — 1.16 gm. It is significant to note that the loss of calcium was diminished during the thirty-sixth week when a daily administration of 15 gm. of cod liver oil and 10 gm. of yeast had been given for the preceding 2 months (Donelson, Nims, Hunscher and Macy, '31).

The acid-base mineral balances of lactation in the present study when calculated by the usual method showed a mean intake of 1350 ± 292 cc. 0.1 N excess base followed by a mean retention of 471 ± 423 cc. 0.1 N excess base per day (table 4). To our knowledge these data represent the initial acid-base mineral balances in human lactation.

One of the most interesting observations in this continued metabolic study occurs in the chemical exchange in pregnancy as contrasted with lactation when there is a decrease from about 0.400 gm. of calcium in the urine daily during the intrauterine development to about 0.065 gm. after the thirty-third day of lactation. These findings corroborate previous observations on the same subject and on two other women in early lactation (Donelson, Nims, Hunscher and Macy, '31).

It has been demonstrated that uncomplicated pregnancy represents a period of appreciable maternal gain over and beyond the fetal requirements (Hunscher, Hummel, Erickson and Macy, '35). Such a maternal reserve or 'rest material' is, in all probability, nature's means of preparing the mother

to carry on the physiological functions of the reproductive cycle without undue burden to her own body. It will be recalled that continuous observation during the final 145 days of gestation showed that L.R., on a generously chosen diet stored a total of 52.90 gm. of calcium, approximately 24 gm. of which were laid down in the fetus thus leaving a known reserve of 29 gm. for utilization or dissipation in the completion of the fourth reproductive cycle. Although the continuous balances were extended only from the tenth to fifty-third days postpartum during a period of heavy milk flow in which there was an observed maternal calcium loss of 21 gm. there still remained a known reserve of 7.90 gm. of calcium. As has been previously emphasized this woman laid down an abundance of the various inorganic elements during the last half of gestation over and beyond what was needed for the fetus and she had none of the common disturbances characteristic of this period. She experienced an excellent preparation through a building up of a generous maternal reserve in pregnancy which enabled her to complete the physiological functions of reproduction even to the secretion of a large quantity of breast milk with a physiological drain for which she was prepared. During the 43 days of lactation there was similarly a gain or loss of —1.57, + 22.27, + 34.83, + 16.55, — 15.80 and — 8.70 gm. of magnesium, sodium, potassium, chlorine, phosphorus and sulfur, respectively. If a mother is organically fit and she is given adequate medical care and advice during the entire reproductive cycle she should be able to carry out the ordinary functions of motherhood without a physiological burden to her own tissues, and at the same time endow her offspring with its rightful heritage—physical and nervous stability.

SUMMARY

Using the continuous metabolic balance method of study on one woman in her fourth reproductive cycle when consuming a generous food intake the mean daily balances of calcium, magnesium, sodium, potassium, phosphorus, sulfur and

chlorine were found to be 0.37 ± 0.19, 0.11 ± 0.05, 0.56 ± 0.53, 1.40 ± 0.72, 0.26 ± 0.16, 0.34 ± 0.13 and 0.60 ± 0.96 gm., respectively, during the final 145 days of pregnancy and -0.48 ± 0.56, -0.03 ± 0.15, $+0.47 \pm 0.46$, $+0.87 \pm 1.06$, -0.37 ± 0.24, -0.22 ± 0.18, $+0.39 \pm 0.88$ gm., respectively, from the tenth to fifty-third days of milk flow.

The twenty-eight acid-base balances during pregnancy showed a mean daily retention with standard deviation of 347 ± 246 cc. 0.1 N base. In lactation, because of losses of acid-forming elements, the base balance increased to 471 ± 423 cc. 0.1 N base per day.

The quantitative determination of the total accumulation of each individual element during the last half of pregnancy permitted a study of the comparative rates of gain of the elements and their relationships to one another with the advance of the reproductive cycle.

By the continuous observations there was no apparent change in the progressive gestatory needs as term approached.

On a maternal diet that was not only abundant in all the known nutritive essentials including minerals and vitamin D but in the proportions to be conducive to a high maternal storage the present woman was able to bear and breast feed an infant who showed no clinical or roentgenographic manifestations of rickets (Barnes, Cope, Hunscher and Macy, '34) although it never received any direct administration of vitamin D up to the eighth month of life other than that contained in its own mother's milk.

Providing a generous maternal reserve has been laid down in pregnancy over and beyond that necessary for fetal growth, the losses of the various elements that do occur in early lactation may have no apparent serious consequences as illustrated by this woman who has undergone frequent reproductive cycles during a period of 8 years.

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THE SIGNIFICANCE AND ACCURACY OF BIOLOGICAL VALUES OF PROTEINS COMPUTED FROM NITROGEN METABOLISM DATA

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Since the introduction by Thomas ('09) of a method of measuring the biological value of protein from nitrogen metabolism data obtained with human subjects, and the modification of this method and its adaptation to rats by Mitchell ('24 a), such methods have been extensively used in the study of the nutritive values of the proteins of food materials. A review of this work up to 1929 has been prepared by Mitchell and Hamilton ('29), and a more recent review was published by Boas-Fixsen ('35). Each investigator has generally introduced more or less extensive modifications of his own, either in technic, in method of calculation, or in application to animals of different age or species. Although in general the results obtained in these various laboratories have been mutually consistent, occasionally a wide variability has been observed among biological values obtained on the same food material (Chick, Boas-Fixsen, Hutchinson and Jackson, '35; Mason and Palmer, '35).

Many observers have commented on the shifting level of endogenous nitrogen excretion observed in rats upon which two or more determinations have been made, while some uncertainty as to the period of feeding required to reduce the protein catabolism to the endogenous level undoubtedly has arisen, particularly since the publication of the work of Ashworth and Brody ('33) on this question. The reality of a

level of endogenous nitrogen excretion possessing a definite biological significance, in particular a definite relationship to the demands of the body for nitrogenous compounds for the maintenance of the integrity of its tissues, is evidently a matter of doubt in the minds of many.

The factors determining the excretion of metabolic nitrogen in the feces are not generally agreed upon, in particular whether this excretion varies mainly in response to variations in the intake of dry matter, or to variations in body weight of the animal, or whether it is an individual characteristic of an animal, unaffected by body weight or food intake.

In defense of the theory underlying the nitrogen metabolism method of determining biological values of proteins, the work of Terroine and his associates (cited fully by Smuts) and of Smuts ('35) possesses great significance. This work reveals a remarkably close correlation between the endogenous excretion of nitrogen as ordinarily determined and the basal heat production, not only within a single species, but also among species varying in weight from the mouse to the pig. According to Smuts, an average of 2 mg. of endogenous nitrogen is excreted per calorie of basal heat. The reality of a constant and significant basal output of nitrogen would thus seem to be as well established as that of a constant and significant basal output of heat. And while conditions, both internal and external, are known to cause a shift in the value of the former, the situation is quite the same with reference to the latter; in particular close and prolonged confinement of an animal will lower its basal metabolism (Lusk and DuBois, '24; Mitchell and Haines, '27). Furthermore, Benedict ('28) has developed the thesis that the basal metabolism is a measure of vital activity and is subject to all those influences that depress or stimulate vital activity.

The factors to which the excretion of metabolic nitrogen in the feces is subject have been studied in some detail by Schneider ('35) and Mitchell ('34). It seems clear that, for levels of food intake compatible with a good determination of biological value, the predominant factor is the quantity of

dry matter consumed. The failure of other investigators to observe this relationship is not surprising whenever the experimental plan involves short collection periods with no use of feces markers.

The variability of individual determinations of the biological value of a protein is the resultant not only of technical error in method, but also of individual differences in protein utilization in metabolism. In thirty of the most recent determinations at this laboratory, involving groups of nine or ten rats each, the average variability, as measured by the standard deviations of the thirty groups of values, is 3.7. The individual standard deviations varied from 1.7 to 7.2, and tended to be larger for the smaller biological values. One may predict, therefore, that the average of ten biological values would possess a standard deviation of 1.2 ($3.7 \div \sqrt{10}$), and that successive averages of ten would in all probability fall within an interval of $\pm$ 2.4 from the grand average of all. The best test of this prediction is afforded by the experiments of Mitchell and Carman ('26), in which five determinations of the biological value of the proteins of patent white flour were made with five groups of ten rats each. The average group determinations were 52, 53, 51, 49 and 55, averaging 52. All averages fell within the interval 52 ± 3. This is a relatively low biological value. The proteins of dried skim milk have a much higher value, which has been assessed at 85 (Mitchell, '24 b), 84 (Mitchell and Carman, '26), 84 (Mitchell, Beadles and Keith, '26) and 82 (Fairbanks and Mitchell) in various publications from this laboratory. These instances illustrate, for a protein of low biological value as well as for one of high value, the reproducibility of results obtained by the nitrogen balance method as it is used in the Division of Animal Nutrition of the University of Illinois.

In this paper the authors will report the results of tests of this method to determine whether the biological values obtained by it possess an absolute significance, or at most merely a comparative significance, as they would if the reasoning upon which their calculation is based is unsound, and if

the significance attached to the endogenous urinary nitrogen and the metabolic fecal nitrogen is false. An illustration will also be given of the significance of small differences between average biological values.

The tests involve a comparison of biological values by the nitrogen balance method and of results of paired-feeding tests with the same rations. It seems unnecessary to give the details of the experimental technic in each case, since they may be found in many previous publications from this laboratory, for example, the paper of Beadles, Quisenberry, Nakamura and Mitchell ('33). The only deviations from past practices were, first, the use of rations containing 22 instead of 10 per cent of fat, in the hope that the change in consistency would be such as to minimize scattering by the experimental rats. Since this hope was not realized, all food portions after being weighed into the food cups were mixed with water to a thin consistency, an expedient that had the desired effect. In previous work, a level of 8 per cent of dietary protein has been adopted, but with the higher fat content of the diets, the protein content was raised from 8 to 9.2 in order to maintain constant the percentage (7.6) of protein calories in the diets. The second deviation from the usual procedure was the use of feces markers, either Fe_2O_3 or Cr_2O_3, to insure sharp separations of fecal collections.

RESULTS OF THE TESTS OF METHOD

I. Beef and peanut proteins

A comparison of the nutritive value of the proteins of raw peanuts (white meat only), and of the same sample of peanuts after roasting (heating to 400° to 450°F. for 30 to 35 minutes), by both the paired-feeding method and the nitrogen balance method, revealed a statistically significant, but numerically small, impairment due to heating. In the former experiment, ten pairs of rats, ranging in weight from 32 to 58 gm., were used. The feeding period lasted 5 weeks, during which time the rate of growth ranged from only 3 to 7 gm. weekly, due to the unpalatability of the raw peanut ration. After the termination of the experimental period, it was shown, by

doubling the daily supplements of yeast vitamin powder for five of the ten pairs, that the low food intakes were not the result of a deficiency of vitamin B or G.

The differences between pair mates with reference to total body weight gains during the 5-week test ranged from 0 to 4; in one pair the rat on the roasted peanut ration gained 1 gm. more than its pair mate, in two pairs the gains were equal, while in seven pairs the rat on the raw peanut ration gained more than its pair mate. These differences in outcome, while numerically small, possess a high statistical significance. Applying Student's method ('08) for the statistical analysis of

TABLE 1

True digestibility and biological value of the proteins of raw and roasted peanuts

RAT NO. AND SEX	RAW PEANUT PROTEIN		ROASTED PEANUT PROTEIN	
	True digestibility	Biological value	True digestibility	Biological value
1 ♂	97.7	61	95.8	55
2 ♂	99.2	60	98.0	54
3 ♂	96.1	56	94.4	47
4 ♂	95.9	63	96.8	59
5 ♀	97.2	56	97.1	51
6 ♀	99.5	63	96.2	61
7 ♀	98.6	59	96.5	57
8 ♀	97.2	56	95.0	54
9 ♀	96.6	44	95.0	62
10 ♀	95.9	61	96.7	58

paired experimental observations, the mean difference in gain is 1.80 gm., favoring the raw peanut protein, the standard deviation of differences is 1.72 gm., and the probability that fortuitous factors alone would produce as consistent a series of differences as those obtained is only 0.006. This probability is so small it may be neglected. The conclusion appears to be justified, therefore, that roasting has impaired the nutritive value of the peanut to a slight extent.

This impairment may relate to digestion or metabolism or both. A further analysis of the problem was made possible by the results of the nitrogen balance studies. The coefficients of true digestibility and the biological values obtained for the ten rats used in these studies are summarized in table 1. The

average coefficients of true digestibility (allowance being made for all metabolic products in the feces) for raw and roasted peanuts are 97.4 and 96.1, respectively, and the average biological values of the absorbed protein are 57.8 and 55.7, respectively. While these averages do not differ greatly, the differences are, with two exceptions in the first case and one in the second, consistent among the ten rats. Analyzed by Student's method, the probability that chance only determined the outcome is 0.0094 ($M = 1.24$, $s = 1.29$) with reference to digestibility, and 0.00005 ($M = 2.1$, $s = 0.99$) with reference to biological value. It may be concluded, therefore, that the impairment of the growth-promoting value of peanut protein during roasting, indicated in the paired-feeding test,

TABLE 2

True digestibility and biological value of beef round protein

RAT NO. AND SEX	TRUE DIGESTIBILITY	BIOLOGICAL VALUE	RAT NO. AND SEX	TRUE DIGESTIBILITY	BIOLOGICAL VALUE
1 ♂	100.0	79	6 ♀	100.0	81
2 ♂	98.0	77	7 ♀	100.0	79
3 ♂	100.0	75	8 ♀	100.0	79
4 ♂	99.4	81	9 ♀	99.5	77
5 ♀	100.0	76	10 ♀	99.3	73

relates both to digestion and to metabolism, although in both instances the impairment is numerically slight. The average biological value for raw peanut protein is in close agreement with the value of 59 obtained by Pian ('30) with six rats.

Immediately after the balance periods on raw and roasted peanuts, the ten rats were put upon a diet containing dried extracted beef round as the predominant source of protein. The results of this period are assembled in table 2. The average coefficient of true digestibility of the beef protein was 99.6 per cent, with six rats giving evidence of complete digestibility. The average biological value of the absorbed protein was 77.7, a value 34 per cent higher than that for raw peanut protein. In a previous publication from this laboratory, a biological value of 69 (Mitchell and Carman, '26) has been

reported for beef protein. However, such a variation is to be expected for meat proteins, because of a variable content of connective tissue proteins (Mitchell, Beadles and Kruger, '27).

A growth comparison of the value of beef protein and the protein of roasted peanuts was undertaken by the paired feeding method, using eight pairs of rats. These rats ranged in weight initially from 30 to 47 gm. and were paired with the usual care. At the end of the first week of feeding the superiority of the beef protein was clearly evident, and at the end of the third week, the advantage in total gain in weight averaged 8.75 gm. and was evident in each pair.

In order to obtain a quantitative measure of this superiority, which is impossible as long as the body weights of pair mates are unequal, the experimental procedure was changed starting with the fourth experimental week. The food intakes of pair mates were still equalized, but the beef protein ration was fed with varying proportions of a diet containing the same ingredients except that the dried extracted beef was replaced by starch. The proportion of this very low-nitrogen diet fed in varying amounts to the rats on the beef diet was increased until the rats on the peanut diet attained approximate weight equality with their pair mates, a result that was attained by the end of the fifth week, and then was continually readjusted to maintain this equality through the twelfth week of feeding, at about which time the experiment was terminated. The significant results of this modified paired-feeding test will be found in table 3.

The total gains in weight of the pairs varied widely for the 12 weeks of feeding, from 40 and 46 gm. for pair 7 to 119 and 112 gm. for pair 2, in close conformance with the variation in the total intake of food, but for the rats upon the same diet the gains were quite similar, averaging 80.5 gm. for the rats on the peanut diet and 81.9 gm. for the rats on the beef diet. The intake of food was identical for all pair mates, but the intake of nitrogen was consistently higher for the rats on the peanut protein, averaging 9.44 gm., as compared with 6.61

gm. for the rats on the beef protein. The ratio of nitrogen intakes of pair mates varied from 1.29 gm. of peanut nitrogen to 1 gm. of beef nitrogen, to 1.74 to 1. The average ratio was 1.46 to 1. This ratio means that 1 gm. of beef nitrogen in this experiment was as effective in promoting the functions of

TABLE 3

A comparison of the growth-promoting value of the proteins of roasted peanuts and of beef round by a modified paired-feeding method.
All weights expressed in grams

	PAIR 1 ♂		PAIR 2 ♂		PAIR 3 ♂		PAIR 4 ♀	
	Peanut ration	Beef ration + N-free ration	Peanut ration	Beef ration + N-free ration	Peanut ration	Beef ration + N-free ration	Peanut ration	Beef ration + N-free ration
Total gain in weight	64	71	119	112	91	91	96	96
Total food consumed	621	621	822	822	705	705	745	745
Total nitrogen consumed	8.77	5.41	11.20	8.71	9.89	7.53	10.51	7.48
Ratio of nitrogen intakes	1.62	1	1.29	1	1.31	1	1.41	1
Activity, revolutions (× 1000)	376	561	340	274	481	869	413	240
Body length in millimeters	184	181	204	203	192	190	190	187
	PAIR 5 ♀		PAIR 6 ♀		PAIR 7 ♂		PAIR 8 ♂	
	Peanut ration	Beef ration + N-free ration	Peanut ration	Beef ration + N-free ration	Peanut ration	Beef ration + N-free ration	Peanut ration	Beef ration + N-free ration
Total gain in weight	97	97	5	56	40	46	82	86
Total food consumed	723	723	538	538	563	563	664	664
Total nitrogen consumed	10.20	7.63	7.60	5.53	7.94	4.56	9.38	6.01
Ratio of nitrogen intakes	1.34	1	1.37	1	1.74	1	1.56	1
Activity, revolutions (× 1000)	397	317	543	1067	1074	560	1029	468
Body length in millimeters	190	188	166	165	172	167	189	184

maintenance and of growth as were 1.46 gm. of peanut nitrogen.

A part of this difference is the result of the poorer digestibility of peanut nitrogen as compared with beef nitrogen, the coefficients of true digestibility being 96.1 and 99.6, respectively (see above).[1] But, on the other hand, the metabolic

[1] The digestibility of the energy of the peanut and beef rations was very nearly the same, averaging (for four rats in each case) 96.9 and 97.8, respectively.

nitrogen of the feces excreted per gram of dietary nitrogen was greater for the rats on the beef ration, because of the lower percentage of nitrogen in the latter diet. When these two factors are taken into account, the latter factor by evaluating the metabolic fecal nitrogen at 1.34 mg. per gram of dry matter consumed (on the basis of the nitrogen balance studies), the ratio expressing the nutritive equivalence of peanut nitrogen and beef nitrogen is practically unchanged, being 1.47 to 1.

In spite of the equality of gains between pair mates, there was a slight, though statistically significant, difference with respect to body length, measured from nose to root of tail, as the figures in table 3 show. In each pair the final body length of the rat on the peanut ration was greater than the body length of its pair mate on the beef protein ration. The average difference amounted to 2.75 mm. while the standard deviation of differences was only 1.48 mm. The probability that chance alone was responsible for this outcome is so small (0.0009) that it must be considered a dietary effect.

When the experiment was planned it was realized that if a large difference in the nutritive values of peanut protein and of beef protein existed, a differential effect of the two diets upon the muscular activity of the animal may occur. In unpublished experiments, one of us (H.H.M.) has shown that the mere restriction of the food intakes of a rat will almost invariably increase its voluntary activity as measured in a revolving cage. If the rats on the peanut protein ration were more active than their pair mates, they would gain less per unit of food energy consumed. Consequently, the feeding experiment was conducted in a battery of sixteen revolving cages, with a revolution counter for each cage. The total revolutions for each rat for the entire experiment are given in table 3, expressed in thousands. There was no consistent relation between the activity of pair mates on the two experimental rations, the average number of revolutions being 582,000 for the rats on the peanut ration and 545,000 for the rats on the beef ration. It may be concluded, therefore, that the results

of the growth experiment were not vitiated in any way by a differential effect of the diets upon voluntary activity.

Although it is commonly believed that equal gains on equal intakes of food denote equal nutritive effects, this is not necessarily true, since the tissue added during growth may vary in composition, particularly with reference to the content of moisture, fat and protein. The reality of this supposition has been proven in other experiments (Beadles, Quisenberry, Nakamura, Mitchell, '33; McClure, Voris and Forbes, '34).

TABLE 4

The contents of nitrogen and energy in the carcasses of four pairs of rats used in comparing the growth-promoting value of peanut protein and beef protein

PAIR NO.	RATION	EMPTY BODY WEIGHT, GM.	NITROGEN CONTENT		ENERGY CONTENT	
			In per cent	In grams	Per gram calories	Total calories
1	Peanut	120.8	2.99	3.61	1.91	231
	Beef	124.8	2.47	3.08	2.82	352
2	Peanut	176.8	2.86	5.06	2.36	417
	Beef	171.1	2.91	4.98	2.52	431
3	Peanut	142.5	3.04	4.33	2.27	323
	Beef	142.6	3.12	4.45	1.97	281
4	Peanut	144.8	3.11	4.50	2.26	327
	Beef	144.6	2.92	4.22	2.29	331

In the present test it seemed advisable to investigate this possibility, and accordingly the first four pairs of rats were subjected to analysis to determine their contents of nitrogen and of gross energy (heat of combustion). The results are summarized in table 4.

There are no consistent differences between pair mates, either with respect to nitrogen or energy content, and, with the unexplained exception of pair 1, there are no great differences between pair mates. These analyses, therefore, afford no grounds for doubting that the two dietary regimes compared in this test actually did produce identical nutritive effects as well as identical gains in body weight.

It may be concluded, therefore, that this modified paired feeding experiment has provided valid evidence that, in the experimental rations fed, 1 gm. of absorbed beef nitrogen has proven to be the nutritive equivalent of 1.47 gm. of absorbed peanut nitrogen in replacing endogenous losses of nitrogen (maintenance) and in promoting growth. The nitrogen balance method yielded biological values for these two mixtures of protein of 77.7 and 55.7, respectively. These values indicate that 1.29 gm. ($1 \div 0.777 = 1.29$) of beef nitrogen cover the body's requirement for 1 gm. of nitrogen, while for the same purpose 1.80 gm. ($1 \div 0.557 = 1.80$) of peanut nitrogen are required. Hence, by these tests 1 gm. of beef nitrogen is the equivalent of 1.40 gm. ($1.80 \div 1.29 = 1.40$) of peanut nitrogen, an equivalence ratio approximating closely to that yielded by the paired feeding technic, i.e., 1 to 1.47 gm. The somewhat greater relative value of beef nitrogen indicated by the latter method may be the result of an enhanced biological value at the lower level of feeding employed, i.e., 0.97 per cent, as compared with 1.40 per cent for the peanut nitrogen. The remarkable agreement between the two technics for assessing the nutritive value of proteins supports the validity of both, and particularly supplies striking confirmation of the somewhat controversial theory upon which the calculation of biological values rests.

II. Beef and pecan proteins

A somewhat similar comparison of the results of the paired-feeding and the nitrogen balance methods was made using beef proteins and pecan proteins. Preliminary digestion experiments indicated a marked difference in the true digestibility of these protein mixtures, beef protein being digested practically completely, while pecan protein was digested only to the extent of about 70 per cent. Hence, the experimental rations were made to contain the same percentage of digestible protein, 9.4 per cent. The gross energy contents of all rations were equalized at 4.85 calories per gram. However, the digestible energy content of the beef ration was 4.66

calories per gram, while that of the pecan ration was 4.32 calories per gram.

In the nitrogen balance experiment the standardizing ration, containing 4 per cent of whole egg, was fed in the second period, the test rations in the first and third periods. Five of the ten rats received the beef ration in period 1, and five the pecan ration. In period 3 the test rations were reversed for the two groups of rats. The rats were all males and weighed initially from 53 to 65 gm. Feces markers, ferric or chromic oxides, were used to separate the period collections.

TABLE 5

True digestibility and biological value of the proteins of round beef and pecan nut

RAT NO.	BEEF ROUND PROTEINS		PECAN NUT PROTEINS	
	True digestibility	Biological value	True digestibility	Biological value
17	100.0	66	73.2	61
19	100.0	71	70.3	63
21	100.0	71	66.3	58
23	100.0	75	70.5	61
25	100.0	75	71.6	64
18	100.0	80	72.9	58
20	100.0	80	71.7	60
22	100.0	78	70.3	59
24	100.0	73	67.7	57
26	100.0	77	72.6	57
Average	100.0	75	70.7	60

The results of the test are summarized in table 5. No evidence was obtained of the excretion of beef nitrogen in the feces of any of the ten rats, so that a coefficient of true digestibility of 100 was assigned in each case. The pecan protein again showed a low true digestibility, averaging 70.7 per cent. The biological values averaged 75 for beef protein and 60 for pecan protein. These values indicate that 1.33 gm. ($1 \div 0.75 = 1.33$) of digestible nitrogen from beef cover the body's requirement for 1 gm. of nitrogen, while for the same purpose 1.67 gm. ($1 \div 0.60 = 1.67$) of nitrogen from the pecan nut are required. Hence, 1 gm. of absorbed beef nitrogen is the nutritive equivalent of 1.26 gm. ($1.67 \div 1.33 = 1.26$) of absorbed pecan nitrogen.

In the growth experiment, eight pairs of rats weighing initially from 50 to 68 gm. were used. During the first 4 weeks of the test, the two rations were fed in equal amount to the two rats in each pair. At the end of this time a distinct difference in gain between pair mates in favor of the beef ration was noted, the mean difference being 7.6 gm., the standard deviation of differences 2.40 gm., and the probability of a fortuitous outcome less than 0.0001. Since the content of protein rather than of digestible energy was the factor limiting growth in both rations, and since the digestible nitrogen content of both rations was the same, this difference in gain may be attributed to the superior biological value of beef proteins.

From the end of the fourth week of the test to the end of the experiment the beef ration was diluted with a nitrogen-free ration until the body weights of pair mates were equalized, the total food intake of pair mates being kept the same. This equalization of body weights of pair mates was accomplished in 2 weeks for six pairs of rats, and in 5.5 weeks for two pairs. At the end of the experiment all rats were killed with ether, the contents of the alimentary canal were removed and the empty carcasses weighed, frozen solid, ground finely in a hand mill and analyzed for nitrogen. The gross energy content of four pairs of rats was determined with the bomb calorimeter. The results of these analyses are given in table 6.

Although the weights of pair mates were equalized in the last weeks of the experiment, the empty weights of the rats on the pecan ration were found to be smaller in all pairs than the weights of the corresponding rats on the beef ration. The percentages of nitrogen in the carcasses of pair mates, as well as the contents of energy in the four pairs examined, averaged almost the same. However, the absolute weights of nitrogen in the carcasses of beef protein rats exceeded those of the carcasses of the pecan protein rats in seven of the eight pairs, the average difference amounting to 77 mg. of nitrogen.

The final calculations from the paired-feeding experiment are given in table 7. The net intake of nitrogen of each rat is estimated by subtracting from the intake of digestible nitrogen the metabolic nitrogen of the feces, assessed at 1.49

TABLE 6

The contents of nitrogen and energy in the carcasses of rats used in comparing the growth-promoting value of beef protein and pecan protein

PAIR NO.	RATION	EMPTY BODY WEIGHT, GM.	NITROGEN CONTENT		ENERGY CONTENT	
			In per cent	In grams	Calories per gram	Total calories
1	Beef	99.2	2.94	2.92	....	....
	Pecan	95.8	2.78	2.66	....	....
2	Beef	110.6	2.99	3.31	2.62	290
	Pecan	109.7	3.01	3.30	2.65	291
3	Beef	105.0	3.01	3.16	2.36	248
	Pecan	103.9	3.18	3.30	2.26	235
4	Beef	95.1	2.94	2.80	....	....
	Pecan	92.4	2.94	2.72	....	....
5	Beef	97.0	3.25	3.15	....	....
	Pecan	95.6	3.08	2.94	....	....
6	Beef	101.2	3.06	3.10	....	....
	Pecan	97.2	3.13	3.04	....	....
7	Beef	97.5	3.09	3.01	2.05	200
	Pecan	96.8	3.02	2.92	2.04	197
8	Beef	96.0	3.15	3.02	2.22	213
	Pecan	93.4	3.19	2.98	2.33	217
Averages						
	Beef		3.054	3.059	2.31	238
	Pecan		3.041	2.982	2.32	235

mg. per gram of food, the average result of the standardizing period in the nitrogen balance experiment. A correction is then applied because of the differences in nitrogen content of pair mates at the end of the experiment (see table 6). Assuming that the rats contained 3 per cent of nitrogen at the begin-

ning of the experiment, it appears from average figures that each gram of nitrogen stored required 2.41 gm. of net nitrogen from the beef ration and 3.01 gm. of nitrogen from the pecan

TABLE 7

The nutritive equivalence of the nitrogen of beef round and of the pecan nut, according to the paired-feeding tests

PAIR NO.	RAT NO.	RATION	FOOD INTAKE	DIGESTIBLE NITROGEN	METABOLIC NITROGEN IN FECES	NET INTAKE OF NITROGEN	DIETARY NITROGEN TO CORRECT FOR DIFFERENCE IN NITROGEN RETENTION	CORRECTED NET INTAKE OF NITROGEN	RATIO
			gm.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	
1	1	Beef	244 (17)[1]	3.76	0.39	3.37	0	3.37	1
	2	Pecan	261	4.08	0.39	3.69	+ 0.78	4.47	1.33
2	3	Beef	331 (101)	5.10	0.69	4.41	0	4.41	1
	4	Pecan	432	6.66	0.69	5.97	+ 0.03	6.01	1.36
3	5	Beef	323 (106)	4.97	0.68	4.29	+ 0.34	4.63	1
	6	Pecan	429	6.63	0.68	5.95	0	5.95	1.29
4	7	Beef	229 (12)	3.53	0.36	3.17	0	3.16	1
	8	Pecan	241	3.77	0.36	3.41	+ 0.24	3.64	1.15
5	9	Beef	221 (21)	3.40	0.35	3.05	0	3.05	1
	10	Pecan	242	3.79	0.35	3.44	+ 0.63	4.07	1.33
6	11	Beef	241 (28)	3.71	0.38	3.33	0	3.33	1
	12	Pecan	269	4.21	0.38	3.83	+ 0.18	4.01	1.20
7	13	Beef	238 (10)	3.66	0.38	3.28	0	3.29	1
	14	Pecan	248	3.88	0.38	3.50	+ 0.27	3.78	1.15
8	15	Beef	243 (17)	3.74	0.39	3.35	0	3.36	1
	16	Pecan	260	4.08	0.39	3.69	+ 0.12	3.81	1.13
Averages		Beef rats				3.53			1
		Pecan rats				4.19			1.24

[1] Amount of nitrogen-free ration consumed.

ration. The correction represents an addition to the net intake of nitrogen of the pair mates containing the smaller final content of nitrogen equal to its deficit of nitrogen multiplied

by 3.01, in the case of a rat on the pecan ration, or by 2.41, in the case of a rat on the beef ration. The corrected net intakes of nitrogen, given in the next to the last column of table 7, would presumably induce equal storages of nitrogen in growing rats. The ratios of these intakes, taking the beef ration rat as a base, are given in the last column. These ratios average 1 to 1.24, and represent the nutritive equivalence of beef and pecan nitrogen.

This average ratio is very close to that obtained in the nitrogen balance studies, i.e., 1 to 1.26. Since the calculations from the growth experiment do not involve any assumptions concerning the magnitude, the constancy, or the significance of the endogenous losses of nitrogen from the body, while those from the nitrogen balance experiments are based in large part upon what appear to be reasonable assumptions in regard to these losses, the approximate identity of the results of both methods constitutes a striking confirmation of the accuracy of these assumptions. Hence, it may be concluded that the biological values determined by the nitrogen balance method possess an absolute as well as a relative significance.

CONCLUSIONS

Biological values of a given protein, as determined by the nitrogen balance method as it is used in this laboratory, exhibit an average standard deviation of 3.7. This statistic tends to be larger the smaller the biological value.

The statistical significance of a difference in biological value between two protein mixtures of less than 4 per cent may be clearly demonstrated by the nitrogen balance method.

The relative nutritive equivalence of two protein mixtures for maintenance and growth is practically the same when evaluated by the nitrogen balance method or by the paired-feeding method supplemented by carcass analyses. Hence, the assumptions underlying the calculation of biological values by the method developed in this laboratory are substantially correct, and the biological values possess an absolute as well as a relative significance.

The biological value of beef round protein, providing 7.6 per cent of the calories of the test ration, averages 76, that of raw and roasted peanuts 58 and 56, respectively, and that of the pecan nut, 60. Roasting of the peanut according to the usual commercial practice depresses both the digestibility and the biological value of its protein, though only to an inappreciable extent.

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RICKETS IN RATS

XV. THE EFFECT OF LOW CALCIUM-HIGH PHOSPHORUS DIETS AT VARIOUS LEVELS AND RATIOS UPON THE PRODUCTION OF RICKETS AND TETANY [1]

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ONE FIGURE

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The process of deposition of minerals in osteoid tissue is usually called calcification, and it was quite natural, therefore, that early investigations focused upon calcium metabolism and calcium deprivation. The study of E. Voit, in which puppies were fed horse meat may be mentioned as an example. Such work antedated both the classic description of the histologic pathology of rickets and the knowledge of the vitamin factors. A review of this literature is given by Goldblatt ('31). Since the investigations of Mellanby, and McCollum, Park and associates, and Sherman and Pappenheimer, the principal interest has centered upon rickets produced by high calcium low phosphorus diets. These produce an advanced degree of rickets in rats and the histologic pathology and serum values of calcium and inorganic phosphorus closely approach those found in ricketic infants. These diets have been employed almost universally in the studies for vitamin tests.

[1] Read before the American Society of Biological Chemists at Detroit, April, 1935.

Since the advent of vitamin D comparatively little attention has been given to low calcium diets. The Baltimore investigators (McCollum et al., '21, '22; Shipley et al., '22) reported that rickets could be produced in rats by diets low in calcium and moderate in phosphorus, findings which have been confirmed by Park et al. ('23) and Jones and Robson ('33). In fact, they defined this as a second type of rickets similar to that found clinically associated with low serum calcium and tetany. In all these studies it was necessary to have in addition to a deficiency of vitamin D or ultra-violet light, a disproportion between the calcium and phosphorus in the diet. In this respect, then, rickets in rats differed essentially from that found in dogs and infants, in which the dietary Ca/P approximated that found in milk (Ca/P = 1.3).

The normal Ca/P has been defined as from 2/1 to 1/2 and it has been shown that such diets will not cause rickets in rats but will cure rickets after it has been produced. In a previous communication (Brown, Shohl et al., '32) we demonstrated that the ratio of Ca/P alone was inadequate to define the ricketogenic properties of a diet and that the absolute amounts or concentrations must also be given. When the ratio was fixed, rickets was more severe at low levels and less severe or absent at higher levels.

Serum calcium and phosphorus studies by Park, Guy and Powers ('23), Bethke and associates ('32), Kramer and Howland ('32), Brown and associates ('32), have shown that the blood serum values for calcium and phosphorus parallel those of the diet. In the last three of these studies the relation to the dietary levels as well as the ratios is clear. When the Ca/P of the diet is constant, as the absolute amounts ingested increase both calcium and phosphorus of the serum are raised.

The ash content of fat-free bone has been used to give insight into the amount of mineral deposition and thus to give a quantitative value of the degree of rickets. Such an evaluation can be made only roughly by other methods. Bethke et al. ('32) have correlated increase in bone ash with the amounts of calcium and phosphorus intake at a given ratio.

He found slight increases in bone ash with low calcium high phosphorus diets parallel to the increase in salt concentration. But no one has correlated serum values and bone ash with both ratios and levels of intake and histologic pathology in this type of rickets.

Binger ('17) has shown that intravenous phosphate caused a lowered serum calcium and tetany in dogs when the solutions were alkaline or neutral, and low serum calcium but no tetany when they were acid. Salvesen, Hastings and McIntosh ('24) were able to show that high phosphate ingestion caused low serum calcium and tetany. Some infants develop tetany without ever passing through a phase of low phosphorus rickets but show low calcium and normal or increased phosphate in the serum (Gerstenberger et al., '30). In the healing of rickets, infants and rats may develop tetany which is associated with an increased serum phosphate (Shohl et al., '32). The effect of low calcium intakes at various levels and ratios of calcium and phosphorus in the diets of rats upon the production of rickets and concomitant tetany has not been described.

It seemed necessary, therefore, to extend and supplement our former investigations of the high calcium low phosphorus diets to include the low calcium high phosphorus diets and thus in a systematic fashion to complete a survey of the various Ca/P ratios and levels attainable with natural foodstuffs. It was not our purpose to include diets in which the calcium or phosphorus was so low as to occur in traces only. Such calcium or phosphorus starvation requires the use of specially purified ingredients and constitutes a separate problem. Neither was it our purpose to add so much salt that nutritive failure would ensue.

PLAN OF EXPERIMENT

Albino rats from our colony were weaned at 21 days and placed upon the experimental diets at 28 days for a period of 21 days. Four to six animals were fed each diet. The animals were weighed weekly.

Diet. The diet of the breeding and lactating animals was Sherman diet B which consists of two-thirds ground whole wheat and one-third whole milk powder, with ¾ per cent of NaCl. Lettuce and beef liver were given about twice a week. The experimental diets consisted of alterations of Steenbock and Black's diet no. 2965 with the $CaCO_3$ omitted (diet A). When lower phosphorus values were desired Hess and Sherman's modification was used (diet M). The former diet consists of 79 per cent corn, 20 per cent gluten and 1 per cent NaCl, and the latter diet is the same except that corn meal is substituted for ground corn.

The calcium contents of these basal rations were 0.06 and 0.05 per cent, respectively. The phosphorus contents were 0.29 and 0.12 per cent. To these were added appropriate amounts of $CaCO_3$ or KH_2PO_4 or both to obtain the desired ratios and levels shown in table 1. The values have been rounded off to even figures. The calcium was varied from 0.06 to 1.0 per cent and the phosphorus from 0.12 to 2.0. Above this level of phosphorus the salt effect is so great that the animals lose weight and die. These diets vary not only in calcium and phosphorus but also in acidity, carbonate and potassium, and hence in any derived values such as Na/K, K/Ca or CO_3/PO_4.

The acidities are also given in the table calculated according to the method of Sherman and Sinclair, except that the phosphorus is given a valence of 1.8. The acidities of the basal diets are +70 and +12 cc. 0.1 N acid per 100 gm. of diet. The additions of calcium and phosphate cause variations from −365 to +495 cc. The greatest acidities are found in the diets to which most phosphate was added.

The effects of the K/Na or K/Ca have not been shown to be of importance in rickets but have been widely studied in regard to tetany. The conditions selected were those most favorable to the production of tetany, except for the acidity: low calcium, high phosphate, high K/Ca and high K/Na (Seekles and Sjollema, '33). Preliminary experiments (Brown, '30) in which Na_2HPO_4 was used and which did

not involve increased acidity or the last two factors did not produce frank tetany.

Methods. The galvanic electrical reactions were determined weekly (Shohl and Bing, '28). At the end of 21 days on the experimental diet the animals were x-rayed. The use of a suitable rat board (Shohl, '31) permitted as many as twenty pictures in 15 minutes, of unanesthetized animals, without exposure to the operator and with the bones in constant position. The rats were bled to death under light ether anesthesia, and autopsied. The calcium and phosphorus of the pooled serum were analyzed by the method of Fiske and Subbarrow ('25) and Fiske and Logan ('31). When sufficient serum remained, total nitrogen was determined by the Kjeldahl method. The leg bones were dissected free of tissue and the femurs of one leg were weighed, dried, extracted with alcohol and ether, and incinerated to determine the ash content. The average of the individual determinations or the group value was used. The bones of the other leg were reserved for histological examination by Dr. S. B. Wolbach, who has kindly furnished a note on his findings.

The criteria of the effect of the various diets employed were: 1) electrical reactions, 2) blood serum analyses for calcium and phosphorus, 3) the per cent of ash of the fat-free femurs, 4) roentgenograms and 5) histological examination of the bones.

RESULTS

The experiment proceeded with no known errors. The gain in weight in most groups averaged about 4 to 7 gm. per week. In the high salt groups especially with high phosphorus and low calcium the gain was 1 to 2 gm. or absent. In a few animals slight losses occurred. The feces were normal; no diarrhoea occurred with the high phosphorus diets. The animals appeared normal and did not seem unduly nervous.

1. Electrical reactions. No animals were seen in general convulsions of tetany. Two individuals were observed in a slight tremor and one of these in carpopedal spasm. Both of these animals were in the groups fed with diets in which

the Ca/P was 1/4. The amounts of current which caused neuromuscular contraction in the animals which showed tremor were low enough to class the response as tetany. All of the other animals at this ratio, Ca/P = 1/4, or with diets containing still greater amounts of phosphorus showed responses to electric stimuli which must be classed as latent tetany. The effects of Ca/P = 1/2, or lower phosphorus gave

TABLE 1

Effect of varying levels and ratios of calcium and phosphorus of the diet

COMPOSITION OF DIET					BONE ASH [2]	BLOOD SERUM		DEGREE OF RICKETS [3]	
Diet	Ca/P	Calcium	Phosphorus	Acidity		Calcium	Phosphorus	X-ray	Histology
		per cent	*per cent*	*cc. 0.1 N* [1]	*per cent*	*mg. per cent*	*mg. per cent*		
R	2	0.25	0.12	— 78	38	8.8	5.2	++	++
S	2	0.50	0.25	—130	45	7.5	5.8	+	+
X	2	1.00	0.50	—365	46	10.0	7.6	—	Not done
N	1	0.12	0.12	— 18	40	4.8	8.8	++	++
K	1	0.25	0.25	— 30	46	6.0	11.4	+	+
L	1	0.50	0.50	— 65	48	7.6	10.0	—	—
Q	1	1.00	1.00	—195	50	9.7	11.0	—	—
M	1/2	0.06	0.12	+ 12	27	5.0	7.8	+++	+++
F	1/2	0.12	0.25	+ 40	41	5.6	9.0	+	+
G	1/2	0.25	0.50	+ 45	46	6.2	11.6	—	±
O	1/2	0.50	1.00	+ 85	46	9.3	9.3	—	—
U	1/2	1.00	2.00	+ 45	48	8.2	10.6	—	—
A	1/4	0.06	0.25	+ 70	28	4.6	9.4	++	++
B	1/4	0.12	0.50	+105	36	4.8	11.0	++	++
I	1/4	0.25	1.00	+195	47	5.6	11.4	—	—
H	1/4	0.50	2.00	+325	42	...	11.2	—	—
C	1/8	0.06	0.50	+135	35	4.2	10.7	+	++
D	1/8	0.12	1.00	+255	35	4.1	9.8	—	++
P	1/8	0.25	2.00	+305	40	6.3	15.2	—	—
E	1/16	0.06	1.00	+285	32	3.9	7.6	—	++
V	1/16	0.12	2.00	+495	36	3.6	11.6	—	—

[1] Calculated per 100 gm. of diet. + = acid; — = base.

[2] Ash of the fat-free femurs.

[3] Plus signs indicate rickets; minus signs no rickets.

responses which were within normal limits. By this method, no difference could be detected as to the absolute amounts of calcium and phosphorus at the same ratio. If such differences occurred they were within the error of the method.

2. Calcium and inorganic phosphorus of the blood serum. In general, the blood reflects the proportions of calcium to phosphorus in the diet. This has previously been well established by Steenbock, by Bethke and associates ('32), and Kramer and Howland ('32). However, when the data given in table 1 are examined, it is at once seen that not only the ratio but also the levels or concentrations have a definite effect. *As the salt level increases at any ratio both the calcium and inorganic phosphate increase.*[2] In Bethke's study ('32) these effects are minimal but from Kramer and Howland's data ('32), though not analyzed from this viewpoint, the same conclusions must be drawn. This is due to the fact that the former did not vary his concentrations as widely as did the latter.

3. Bone ash. The data given in table 1 also clearly show that both the ratio and level of calcium and phosphorus determine the mineralization of bone. As the phosphorus is increased in proportion to calcium the bone ash is diminished. Furthermore, the effect of the levels is marked. When the concentrations are low the bone ash is low *and as the levels increase at a given ratio the bone ash increases.*[2] Our findings are in agreement with those of Bethke for the diets he used, but unfortunately he did not use diets low enough in calcium and phosphorus to bring out the relations shown here.

4. X-ray. The findings of the x-ray pictures are given in table 1 and graphed in figure 1. For the sake of completeness we have included in the figure the data from our high calcium low phosphorus studies (Brown et al., '32) to cover the entire range of diets available. The shaded area includes all changes from the normal regardless of the degree of rickets. The three types of shadings are simply a convenient separation

[2] If one wishes to take the trouble to rearrange according to levels of Ca and/or P in the diet (not tabled), this effect is brought out more strikingly.

into the rickets produced by high calcium low phosphorus, low calcium low phosphorus and low calcium high phosphorus diets respectively. The unshaded area represents the field in which no rickets was obtained. The degree of rickets indicated by the number of plus signs is only roughly quantitative.

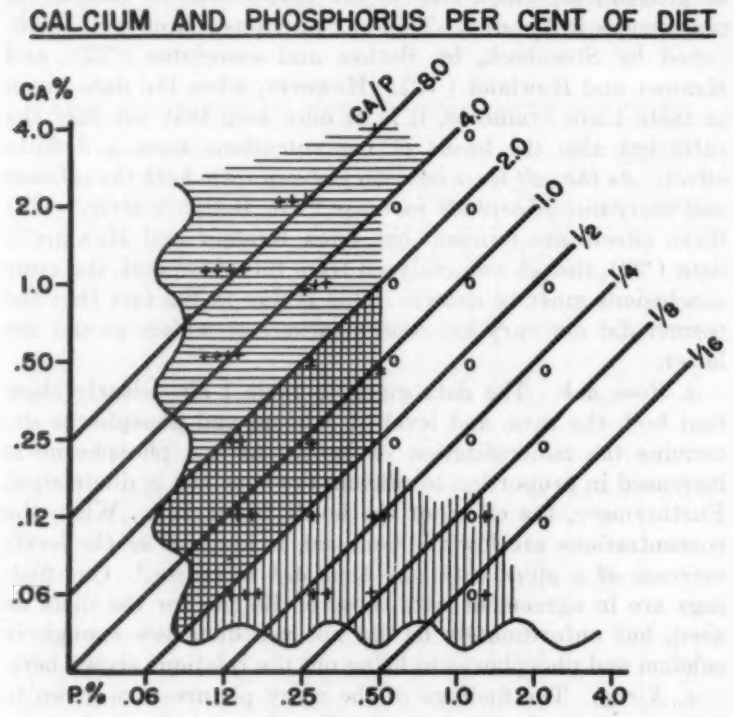

Fig. 1 For explanation see text.

Thus it can be seen that each of these three categories includes diets which are ricketogenic or non-ricketogenic depending upon the absolute amounts of calcium and phosphorus contained.

The graph was drawn independently of the histological findings and the conclusions differ from Doctor Wolbach's interpretation only in regard to the groups fed diets D and E.

These have a very narrow metaphysis, but histologically show rickets. The histological examination places these diets in the ricketogenic group, and the graph shows them in this category.

5. Bone histology.[8] The upper end of the tibia was used for histological study. The technic was fixation in 10 per cent formalin solution, decalcification in 5 per cent HNO_3 in 70 per cent alcohol, celloidin embedding and staining with hematoxylin and eosin. One or more representatives of each diet listed in table 1 were studied, with the exception of diet X, as follows: diets K, L and F, one rat each; diets R, S, Q, G, O, U, B, I, H, C, P, E, D and V, two rats each; diets N, M and A, four rats each.

The important histological criteria of rickets arise from disturbances in endochondral formation of bone and from failure of bone matrix or osteoid to calcify in all locations. For the purposes of this study we have restricted our observations to the epi-diaphysial regions, because most of the bones had been partially denuded of periosteum before fixation.

In brief review, the sequences in enchondral bone formation which are disturbed in rickets are as follows. The epiphyseal cartilage at the upper end of the tibia in normal white rats of the age employed consists of a narrow plate of cartilage firmly supported by bone on the epiphyseal side and uniformly penetrated by blood vessels of capillary dimensions on the diaphysial side. Very little evidence of growth is present on the epiphyseal side where bone is closely applied in the form of transverse trabeculae or a thin fenestrated plate. Growth is accomplished by continuous proliferation of cartilage cells, arranged in columns, on the epiphyseal side and simultaneous degeneration of the matured cells on the diaphysial side. The cavities occasioned by the degeneration and disappearance of the cartilage cells at the diaphysial end of the columns are invaded by capillaries accompanied by cells (osteoblasts) which are responsible for the deposition of bone matrix upon the exposed cartilage matrix.

[8] By Dr. S. B. Wolbach.

Growth of bone by enchondral bone formation is thus achieved by a continuously retreating gap in the continuity of tissues, maintained on the epiphyseal side by continuous renewal of cartilage cells and on the diaphysial side repaired by vascular outgrowth comparable to repair of any defect of tissues by the process of organization or granulation tissue formation. In normal growth there presents on the diaphysial side of the narrow epiphyseal cartilage a continuous layer of clear or empty cartilage cells forming an almost straight line.

The first histological evidence of rickets is the absence in whole or in part of the layer of clear cells and the consequent absence of ingrowth of capillaries. Slight degrees of rickets are manifested by a moderate increase in width of the epiphyseal cartilage presenting an irregular border on the diaphysial side. This irregularity is due to the fact that the cessation of degenerative sequence in the cartilage cells does not take place simultaneously over the diaphysial border. The width of the epiphyseal cartilage continues to increase because of the continued activity of the proliferative zone and the survival of the cells on the diaphysial side. The first histological evidence of repair following corrections of the diet is the presence of cleared or degenerated cells on the diaphysial border of the cartilage, an effect visible at the end of 24 hours and accompanied by extensive vascular penetration within 48 hours (unpublished experiments of S.B.W.).

In rickets, after the cessation of the degenerative sequences of the cartilage cells, calcification of the cartilage matrix ceases, and osteoid material (bone matrix) accumulates around the capillaries of the diaphysis adjacent to the cartilage. In advanced rickets the noncalcified cartilage of the diaphysial border is often transversely stratified in places, evidently a mechanical effort from weight bearing. Osteoid material increases in amount with the duration of the dietary deficiency and, being noncalcified, is molded by the pressure of weight bearing. In long continued rickets there is disappearance of the cancellous bone of the diaphysis and marked

resorption of cortical bone. The degree or severity of rickets may be recorded on the basis of the prominence of anatomical changes demonstrable by roentgenograms or histological study. The latter is the more accurate. Obviously two factors enter into the production of the pathological picture; the duration of the deficient diet and the degree of the deficiency. In the present study the time element was constant so that the extent to which normal sequences were held in abeyance, with consequent increase of width of cartilage and accumulation of osteoid, may be regarded as a roughly quantitative means of measurement of the effect of the rickets producing diet.

No histological evidence of rickets was found in rats fed on diets Q, O, U, I, H, P, V, and in one rat of two examined fed on diet G. The slightest histological effect of rickets manifested by irregular cessation of penetration of the cartilage by capillaries and slight irregularity in the thickness of the cartilage as a whole, was found in the one rat examined fed on diet L.

Slight rickets, characterized by markedly diminished penetration of the cartilage by capillaries, considerable to marked irregular thickening of the cartilage as a whole, persistence of cartilage cells in the primary bone trabeculae (primary spongiosa), no stratification of cartilage and no osteoid was found in rats fed on diets K and B, also in one of two rats examined fed on diet S, and in one of two rats examined fed on diet G.

Moderate rickets, characterized by complete or almost complete absence of penetration of cartilage cells by capillaries, marked thickening of the cartilage as a whole, marked incorporation of cartilage cell groups in the primary bone trabeculae (primary osteoid), traces of stratification of cartilage and small amounts of osteoid were found in rats fed on diets R, N, A and C, also in one of two rats examined fed on diet S, and in one of four rats examined fed on diet M.

Severe rickets, characterized by complete absence of penetration of cartilage cells by capillaries, marked thickening of

the cartilage as a whole, marked incorporation of groups of cartilage cells in the primary spongiosa, marked stratification of cartilage and large amounts of osteoid were found in a rat fed on diet F, and in three of four rats examined fed upon diet M.

The histology found in the rats fed upon diets D and E requires special mention, as the presence of rickets was not demonstrated by the roentgenograms. Two rats were examined from each of these diets. In both instances there was almost complete absence of penetration of cartilage cells by capillaries, marked incorporation of cartilage cells in the primary bone trabeculae (primary spongiosa) and considerable amounts of osteoid present in proximity to the cartilage. In both instances the epiphyseal cartilage was narrow as compared with all other rats with evidences of rickets. In the rats of diet D the width was slightly greater than normal. In the rats of diet E the width was not greater than normal and in one of these it was narrower than the average width found in control rats.

Discussion. The prominent morphological changes in experimental rickets must be regarded as the consequence of retardation of normal sequences in the growth of bone. Two apparently separate processes are involved, one the failure of the epiphyseal cartilage cells to complete their cycle of growth, maturation and degeneration, and the other the failure of osteoid matrix to calcify. The proliferative activities of cartilage cells, capillaries and osteoblasts are not inhibited. The histological picture, typical of rickets, indicates that the proliferative activities of these tissues are not retarded and in some instances suggests acceleration. In the rats fed on diet E the narrow epiphyseal cartilages did indicate a marked retardation of rate of growth of the cartilage cells, but more study will be required to determine whether an interpretation on a purely chemical basis should be attempted.

The histological study of this series clearly proves that the effects of the various diets employed are essentially similar. The differences are wholly quantitative and may be estimated

roughly on the basis of the extent to which normal sequences, essential to bone formation, have been held in abeyance. Histological examination may reveal definite evidences of rickets in the absence of grossly demonstrable changes in the epiphyseal cartilage.

DISCUSSION

The findings in this study, showing the interrelationship of both the ratio and absolute amounts of calcium and phosphorus in the diet of rats to the production of rickets, have been checked against the classic papers in the literature. Without undue citation it may be stated that, with no important exception, the diets fall in the fields as graphed. Thus a number of cases which when considered from the viewpoint of ratio alone seemed contrary to the expected result now fall into the larger and more general scheme. The relation of diet to the production of rickets has thus been clarified.

It is at once evident from histological and x-ray studies in this and the companion study (Brown et al., '32) that rickets may be either present or absent with almost any blood picture. Variations in the serum calcium and phosphorus can be interpreted as a quantitative measure of rickets only when the diet is fixed, as in vitamin testing; or in the rickets of infants, where milk determines the calcium and phosphorus intake. In our experiments the serum calcium values of 3.6 and 3.9 mg. per cent (less than 1 mM.) are as low and the phosphates, above 15 mg. (5 mM.) are as high as we have encountered in dietetic studies. The protein values in scattered analyses are roughly constant at 5.5 per cent and hence the fraction bound by protein is normal and constant. Such material would be especially suited for a study of the forms of calcium in the blood serum by the methods recently published by McLean and Hastings ('35). The ionized and other fractions could then be determined under conditions where the phosphate is the main variable.

The bone ash gives an excellent quantitative insight into the amount of mineral deposition but obviously cannot differentiate between rickets and osteoporosis, between failure to ossify or demineralization. In these studies values below 40 per cent (except at dietary ratio of 1.0, or with very high phosphorus intake) have been associated with rickets. Properly used this gives an excellent insight into the degree of ossification.

From a correlation of the above criteria it is clear that rickets in rats bears a closer relation to the found in infants and dogs than was formely supposed, in that rickets is produced with diets in which the ratio has been considered 'normal,' provided the amounts of calcium and phosphorus are sufficiently low. The essentials for the production of rickets are an inadequacy of vitamin D accompanied by relative deficiency of calcium or phosphorus or an absolute deficiency of either or of both.

Tetany is found associated with low calcium and high phosphate in the serum. Although such blood values were present in the animals made ricketic on high phosphorus low calcium diets, they showed only moderate tetany. But the same blood picture is present when animals with low phosphorus rickets or even normals are changed to high phosphorus diets. These animals respond with tetany of such violence as often to be fatal. This is presumably due to the rapid change in the blood from low phosphate to high phosphate with the rapid deposition of calcium phosphate in the bones.

In the present experiments the highest phosphate diets were not associated with the most marked tetany. The increased acidity of these diets may have been a factor, for it is well known that acid tends to prevent tetany and alkali to produce it. Salvesen, Hastings and McIntosh ('24) reported that when the ingested phosphate was moderate, the neutral salts produced greater effect than the acid or alkaline ones (compare also Shohl et al., '28 a). With larger doses, however, all three produced violent tetany. It is difficult to compare the size of a 50 mg. dose for a 50 gm. rat with a 5.0 to

7.0 gm. dose for a 13 to 20 kg. dog, and undoubtedly both the level of intake and acidity must be carefully evaluated.

In this experiment the principal interest lay in the analysis of rickets and not of tetany, and no further studies in acid-base were carried out. Previous experiments had shown that when severe tetany was present the rats could not or would not eat the food. Under these conditions rickets heals—therefore severe tetany was to be avoided as a complicating factor. The acid-base value of the diet has been shown previously to have a definite effect on the ricketogenic properties of the diet; the acid diets tend toward the production of rickets and the alkaline toward its prevention (Shohl et al., '28 a). In the type of diet used in this experiment the effect is secondary to the calcium and phosphorus relations (Shohl et al., '32). Preliminary experiments had shown that high phosphate diets, when neutral, were not ricketogenic (Brown, '30). In the present study no effort was made to keep the acidity constant throughout, but the acidity was permitted to increase with the KH_2PO_4 additions. This would tend toward increase in the amount of rickets produced, at a given ratio, as the levels increased; but the data show the converse, and hence the conclusion that high salt diets prevent rickets is further enhanced.

CONCLUSIONS

1. Serum calcium and inorganic phosphorus reflect the ratios of the diet. Rickets may be present when the serum phosphate or calcium or both are low. When the calcium is low and the phosphate high, tetany occurs. As the levels in the diet increase, at a given ratio, both serum calcium and phosphate and the per cent of ash of the fat-free bones increase, and rickets and tetany diminish. X-ray, and as a final criterion, the histology of the bones are necessary to form a judgment as to the presence or absence of rickets.

2. From a survey of the whole field of calcium and phosphorus relationship in the diet, in the absence of vitamin D, it is clear that rickets may be produced with not only high

calcium low phosphorus diets, and low calcium high phosphorus diets, but also with low calcium low phosphorus diets. The last group, not previously described, occurs in a zone in which the ratios of Ca/P have been called 'normal,' a designation which has lost its significance, for rickets may be produced with any ratio of Ca/P in the diet. As the absolute amounts are increased, for any given ratio, the diet changes from a ricketogenic to a non-ricketogenic diet.

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ERRATA

In table 2 of the paper on "The variation in the mineral content of vegetables," by Davidson and LeClerc, this journal, volume 11, number 1, page 64, January, 1936, the sulphur figures should read: for spinach, 0.465–0.66 142; for kale, 0.64–1.14 176; for broccoli, 1.15–1.42 123.